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AUGUST KROGH*

NOVEMBER 15, 1874 — SEPTEMBER 13, 1949

P. BRANDT REHBERG

Schack August Steenberg Krogh was born in Grenaa, Jutland. His ancestors had emigrated from Holstein and Schleswig, where his father's family had settled as small farmers three hundred years earlier. Krogh's father was a brewer, though he had been trained as a shipbuilder. Ships and the sea were some of Krogh's unceasing interests.

Krogh, who was the oldest of six brothers and sisters, had already been taught to read and write by his mother at the age of five. Soon after his sixth birthday she wrote in a letter: "August reads almost too fluently, as he frequently out of sheer eagerness omits the signs. We have begun with German, geography and arithmetic. Next fall, if it please God, I shall hand him over to the secondary school in an adequate state; then he himself must take care of his education." Krogh's aversion to official punctuation remained characteristic of him; he put the punctuation marks as it pleased him. After the summer vacation, at the age of six, he was sent to a small secondary school. According to his own opinion, however, going to school did not markedly influence his development. Outside school he cultivated his early awakened interest in nature. At an early age he was interested in animals and for hours he would follow the behavior of insects and spiders. Likewise, with the help of Rostrup's *Flora,* he botanized eagerly in the surroundings of Grenaa. At the age of twelve he had read from beginning to end the large popular Danish encyclopedia available at that time. At this early age he also tried his hand at experimenting, performing—though not always successfully—the chemical and physical experiments he had read about. He had to content himself with the limited material within his reach in the small country town, and he himself assumed that this fact helped to develop his later remarkable ingenuity in improvising apparatus from the few and primitive things at his disposal. As a rule, Krogh's apparatus was inexpensive, and he maintained his economizing attitude even after large funds had been put under his control. Also he exhibited by this time a thorough or rather an exaggerated economy with paper; later in his life he used the tiniest possible scraps of paper, a habit which made it seldom easy for others to decipher his writing.

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* From the Zoophysiological Laboratory, The University of Copenhagen. This appreciation of Professor Krogh first appeared in Danish in the Year Book of the University of Copenhagen. At the request of the Editors, Professor Rehberg very kindly prepared an English translation for this *Journal.*

In 1889, when in his fifteenth year, he became a voluntary disciple in the navy. It was his intention to be a naval officer, partly because he could not advance in school until he was sixteen years old and eligible for the preliminary examinations. However, after an expedition with the inspection boat *Hauch* he renounced this plan, passed the preliminary, and later, in 1893, the final examination at Aarhus cathedral school. Krogh hesitated: should he study physics or choose zoology in which he had taken an interest through his older friend, the zoologist William Sørensen (who spent many holidays in Grenaa and with whom Krogh used to scour the fields)? He passed the university "prelims" and then wrote an extensive outline of his plans. With this he persuaded his father to give him permission to study zoology. At this juncture Krogh was fortunate: William Sørensen, who was ahead of his time in comprehending the general significance of physiology for zoology, suggested that Krogh should attend Christian Bohr's lectures for medical students. Krogh followed Sørensen's advice—a step which turned out to have a decisive influence on his future. After the very first lecture he understood that here was his proper field and, in 1899 when he received his master's degree, he was appointed assistant at Christian Bohr's laboratory of medical physiology at the University of Copenhagen.

However, prior to this appointment, Krogh had begun to devote himself to zoophysiological problems. As a student he investigated the hydrostatic mechanism of *Corethra* larvae, a peculiar type of mosquito larvae which, by means of completely closed air sacs acting as a swim bladder, is able to regulate its buoyancy in water in the same manner as fishes. Krogh did not solve the mystery of their curious organs, but for use in these studies he worked out a method for the microscopic analysis of small air bubbles which, however, was not published until 1911.[1] It is typical of Krogh's first publications that they are based on an understanding of physics which was rarely found in biological circles at that time. He wrote about the significance of turgescence for plants, hydrostatic conditions in the animal kingdom, gases in sea water, and sap rising in plants—frequently in the form of a criticism of the interpretations of other scientists whom he showed were lacking the necessary knowledge of physics. This ability to apply physical knowledge to biological domains remained typical of Krogh's work, which was always distinguished by a brilliant intuitive perception of physical conditions, especially in microscopic dimensions. In contrast, when he was forced to use chemistry in his work, one had the impression that his chemical knowledge was acquired knowledge.

Krogh's ability to find the cause of discrepancies in other investigators' results was well developed at that time and always played an important part in his scientific studies. Many years later, he himself explained his

[1] On the hydrostatic mechanism of the Corethra larva with an account of methods of microscopical gas analysis. Skand. Arch. Physiol., 1911, *25*, 183.

view in the following way: "When experimental results are found to be in conflict with those of an earlier investigator the matter is often taken too easily and disposed of, for instance by pointing out a possible source of error in the experiments of the predecessor, but without inquiring whether the error, if present, would be quantitatively sufficient to explain the discrepancy. I think that disagreement with former results should never be taken easily, but every effort should be made to find the true explanation. This can be done in many more cases than it is actually done; and as a rule it can be done more easily than by anybody else by the man 'on the spot' who is already familiar with essential details, but it may require a great deal of imagination and very often it will require supplementary experiments."[2]

The time spent in Christian Bohr's laboratory became of utmost importance to Krogh through the insight he gained there into the significance of quantitative work and the necessity of proving whether a theory is quantitatively sufficient. Quantitative work was especially attractive to him, the more so when the existing technique was unsatisfactory and accuracy was paramount. This principle, together with physical intuition, was to be typical of much of his work.

Another ability, also of great importance for Krogh, was his capacity for "visual thinking," as he called it. He was able to see before him the image of an apparatus, projected into space; he could preserve this image, improve it, and get it to move. He made use of this ability when he constructed an apparatus, when he scrutinized the descriptions of other investigators' experiments, and when he reflected upon the way in which an organ functions. Krogh never made a drawing before having seen in this way the apparatus ready before him; if he drew at too early a stage he felt hampered. In a paper on "visual thinking" he described this habit of thought which he assumed to be the background of all his other abilities.[3] In this paper he also mentioned that his brain frequently worked unconsciously and he perceived many ideas during sleep. His memory was queerly specialized: animals, plants, and apparatuses were remembered directly while he, for example, was unable to recall special pages in a book or faces. On the other hand, he retained by heart—but purely intellectually—the quotations and verses which enchanted him, while he was unable to recollect chemical formulae.

Another special gift should be mentioned here, namely, that of observation. He was able to detect minute details, for example, in a microscopic picture of living tissue or in a complicated experimental arrangement. Thus, during his study of the capillaries, he worked with much lower

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[2] The progress of physiology. Am. J. Physiol., 1929, *90*, 243; also in Science, 1929, *70*, 200.

[3] Visual thinking. An autobiographical note. Organon, Warsaw, 1938, *2*, 87.

microscopic magnifications than any of his numerous collaborators, but time and again he surprised them by describing the details he could observe at low magnification.

Krogh's first extensive work, his thesis in 1903 on skin and lung respiration of the frog, appeared as a synthesis of his own zoological interests and Christian Bohr's principles of respiration physiology.[4] Here he showed that oxygen metabolism occurs primarily through the thin lung walls with their short diffusion path, while the more easily diffusible carbon dioxide escapes essentially through the skin. The dissertation shows strikingly Krogh's sense for quantitative viewpoints.

Krogh's investigation which was first to gain international renown is a good example of his typical abilities. The Imperial Academy of the Sciences of Vienna had offered a prize for investigations into the participation of free nitrogen in respiratory metabolism. This task required scrupulous accuracy and for a long time Krogh was concerned with it in his thoughts. Finally he arrived at the conclusion that it would be necessary to control the temperature in the respiration apparatus more accurately than usual. Therefore, the whole apparatus should be immersed in water, a procedure which would be feasible only if it were very small. Consequently, Krogh constructed a small apparatus, and it appeared that the results of previous investigators were erroneous because of faulty temperature control. In this investigation his unique capacity for designing apparatus, his critical sense, and his ability to work quantitatively were already fully developed. Krogh demonstrated that nitrogen does not participate in the respiratory metabolism, and he won the offered prize.[5] This treatise is an impressive example of his working method, which he later applied to his long series of investigations into respiratory physiology.

In his doctoral thesis Krogh pointed out that the respiration of toad's skin can be assumed to be due exclusively to diffusion, while, on the other hand, he wholly supported Bohr's views on lung respiration, stating that it occurs predominantly by means of secretory processes and is regulated by the nervous system. However, in the years to come, he became more familiar with respiratory processes and he came to realize that neither in his own works nor in those of other scientists could a valid proof of this view be found. With the methods available, no answer could be given to the serious controversial question of whether the gas exchange in the lungs takes place by a passive or an active process. New apparatus yielding much greater accuracy had to be constructed. The most important one was Krogh's microtonometer with its associated micro gas analysis ap-

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[4] *Frøernes Hud- og Lungerespiration.* København, Gyldendalske Boghandel, 1903, 114 pp.

[5] Experimental researches on the expiration of free nitrogen from the body. Skand. Arch. Physiol., 1906, *18,* 364.

paratus.[6] The microtonometer requires an air bubble of only *ca.* 10 mm.,[3] and, therefore, the equilibrium between air tension in the blood and in the bubble is reached rapidly. By means of this apparatus a small air bubble can be analyzed with sufficient accuracy, and its composition can be compared with the air in the lung alveolus.

Through numerous experiments and a steady pursuit of possible sources of error Krogh finally arrived at the conviction that Bohr was wrong: gas exchange is due to diffusion. This conclusion brought Krogh into a precarious situation. He was Bohr's assistant and at the same time his intimate co-worker. He even had the largest share in designing the apparatus used in the work published in collaboration with Bohr and Hasselbalch on the connection between carbon dioxide tension and oxygen binding of the blood.[7] Therefore he hesitated a long time before publishing his results on the gas exchange in the lungs. However, in 1910, the famous seven papers on "The mechanism of gas-exchange" appeared.[8–14] Before exposing the decisive material in the last paper he lowered the rapier before his old tutor with the following words: "I shall be obliged in the following pages to combat the views of my teacher Prof. Bohr on certain essential points and also to criticise a few of his experimental results. I wish here not only to acknowledge the debt of gratitude which I, personally, owe to him, but also to emphasize the fact, patent to everybody who is familiar with the problems here discussed, that the real progress, made during the last twenty years in the knowledge of the processes in the lungs, is mainly due to his labours and to that refinement of methods, which he has introduced. The theory of the lung as a gland has justified its existence and done excellent service in bringing forward facts, which will survive any theoretical construction, which has been or shall hereafter be put upon them."[14] But then he continues his criticism and gives a review of his own results of tension measurements. The result of these investigations, which has ever since formed the basis of our knowledge concerning the uptake of

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[6] On microanalysis of gases. Skand. Arch. Physiol., 1908, *20*, 279.

[7] Bohr, Christian, Hasselbalch, K. A., and Krogh, August: Ueber einen biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung übt. Skand. Arch. Physiol., 1904, *16*, 402.

[8] Krogh, August and Krogh, Marie: On the tension of gases in the arterial blood. Skand. Arch. Physiol., 1910, *23*, 179.

[9] Krogh, August and Krogh, Marie: On the oxygen metabolism of the blood. Skand. Arch. Physiol., 1910, *23*, 193.

[10] Krogh, August and Krogh, Marie: On the mechanism of the gas exchange in the lungs of the tortoise. Skand. Arch. Physiol., 1910, *23*, 200.

[11] Krogh, August and Krogh, Marie: On the combination of haemoglobin with mixtures of oxygen and carbonic oxide. Skand. Arch. Physiol., 1910, *23*, 217.

[12] Krogh, August and Krogh, Marie: Some experiments on the invasion of oxygen and carbonic oxide into water. Skand. Arch. Physiol., 1910, *23*, 224.

[13] Krogh, August and Krogh, Marie: On the rate of diffusion of carbonic oxide into the lungs of man. Skand. Arch. Physiol., 1910, *23*, 236.

[14] Krogh, August and Krogh, Marie: On the mechanism of the gas exchange in the lungs. Skand. Arch. Physiol., 1910, *23*, 248.

oxygen in the lungs, was summarized by Krogh himself as follows: "The absorption of oxygen and the elimination of carbon dioxide in the lungs takes place by diffusion and by diffusion alone. There is no trustworthy evidence of any regulation of this process on the part of the organism."[14] For some time the advocates of the secretion theory tried to contest Krogh's views, but every check of his results, even when carried out under the most unfavorable conditions, corroborated his findings and served to emphasize the correctness of his statement that the partial pressure of oxygen was always higher in the alveolar air than in the blood; therefore, conditions were favorable for diffusion.

In collaboration with Marie Krogh (he had been married in 1905) Krogh subsequently investigated whether this diffusion was sufficiently large to explain the uptake of oxygen. In her thesis,[15] Marie Krogh applied the method of determining the diffusion constant of the lungs, which had been worked out in collaboration with August Krogh, and she was able to state conclusively that diffusion is sufficient to explain the oxygen uptake. The new view on the gas exchange in the lungs brought forward a series of new problems, and many questions had to be reconsidered and treated on the basis of the recent observations.

In the following years Krogh published numerous papers in a great variety of fields, including the circulation through the lungs, and the significance of the venous fluid for the minute volume of the heart. These investigations were performed at times in collaboration with Marie Krogh, at times with Johs. Lindhard, the medical doctor who later was appointed professor in the theory of gymnastics. Through many years Krogh kept up an extremely fruitful collaboration with both scientists. Like Krogh, Lindhard was very critical and he claimed equally high accuracy in his experiments. He was not endowed with the same imagination as Krogh, but their collaboration was exemplary and resulted in a series of extremely important papers.

Marie Krogh's contribution to the development of certain sides of Krogh's work cannot be estimated highly enough. Throughout his career Krogh worked on two fronts, as a comparative physiologist dealing with a series of zoophysiological problems, and as a specialist in human physiology concerned with a number of physiological problems of paramount significance for medical science. Marie Krogh, who was a physician, no doubt recognized more clearly than did Krogh himself the medical implications of his work and she certainly played an important part in the choice of his subjects during the years when problems of human physiology prevailed over zoophysiological ones.

Krogh left Bohr's laboratory in 1908. In the same year, he was appointed

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[15] *Luftdiffusionen gennem menneskets lunger.* København, Jensen & Kjeldskov, 1914, 123 pp., illus.

lecturer in zoophysiology, although without a laboratory, until in 1910 the "old laboratory," as his pupils called it, was first established. Krogh came to work under modest conditions; the laboratory was installed at the place where his official residence was supposed to be, while he himself moved to a flat in the garret. Nevertheless, the laboratory was equipped according to Krogh's liking, and one could find there many of the laboratory refinements which later reappeared in the new laboratory at Juliane Maries Vej and from there made their way into numerous laboratories all over the world.

During the time before the laboratory was established, Krogh and his wife took the opportunity of making an expedition to the Isle of Disko where they performed a series of studies on the metabolism and diet of Eskimos.[16] This was indeed a task which put a person to the test; but Krogh carried through his experiments even under the most difficult climatic conditions.

After the establishment of the "old laboratory" Krogh continued his work on human physiology. In collaboration with Lindhard, he started a series of investigations on the physiology of exercise, which became models for numerous studies of this type throughout the world and formed the basis for a Scandinavian school within this branch of science. This school can be characterized by such names as Emanuel Hansen, Erik Hohwü Christensen, Marius Nielsen, and Erling Asmussen. Their work was so distinguished that, in 1931, the Health Organization of the League of Nations entrusted the group with the task of performing a model investigation into problems of the physiology of exercise. This task was attacked by a "team" of young scientists, one of the first and typical examples of teamwork which later became so popular; the result was a number of important papers.[17]

In the first investigations on the physiology of exercise during the period 1910-1920, numerous methods for the determination of circulation and respiration were developed. Thus, for example, the minute volume of the heart at work was studied in detail.[18] During these years, Krogh's eminent skill as a designer of apparatus came to its full development; instruments of greatest importance, such as the "tilting spirometer,"[19] the electromagnetic bicycle ergometer,[19] and an apparatus for gas analysis with an accuracy of 1/1000% were designed.[20] By means of these instruments the

[16] A study of the diet and metabolism of Eskimos undertaken in 1908 on an expedition to Greenland. Medd. om Grønland, 1913, *51*, 1.

[17] See Krogh, August: Liste over August Kroghs publikationer. Vid. Medd. Nat. For., 1948-49, *3*, xvii.

[18] Krogh, August and Lindhard, Johannes: Measurements of the blood flow through the lungs of man. Skand. Arch. Physiol., 1912, *27*, 100.

[19] Krogh, August: A bicycle ergometer and respiration apparatus for the experimental study of muscular work. Skand. Arch. Physiol., 1913, *30*, 375.

[20] Krogh, August: A gas analysis apparatus accurate to 0.001% mainly designed for respiratory exchange work. Biochem. J., Lond., 1920, *14*, 267.

influence of food on the efficiency of muscular work was demonstrated,[21] and it was found that in the beginning of muscular work the organism develops an oxygen deficiency which is not compensated for until the conclusion of the work.[22] At the same time, Krogh studied circulation and clearly recognized the importance of the venous flow back to the heart. Finally, he developed, in collaboration with Lindhard, the N_2O-method for the determination of the heart's minute volume.[23]

In connection with these investigations Krogh was automatically led to problems concerning the oxygen supply of the tissue. At the start of physical exercise, a considerable improvement of the blood distribution in the working muscles must occur; otherwise, the better utilization of the oxygen content of the blood cannot be explained. Very early Krogh actually arrived at the conclusion that during rest a large number of muscle capillaries are closed and that they open during work.[24] The problem was now to prove this assumption. Quite new devices had to be applied: microscopic observation of living tissue, injection methods, and histological methods. After intense work, again and again checking and rejecting the methods, Krogh succeeded in showing that his conclusion was correct.[25] In the course of 1918-1919 his results were published in scientific journals and immediately aroused greatest attention. Surprisingly soon the great significance of Krogh's observations was understood and by 1920 he was awarded the Nobel prize in medicine and physiology. It has been objected that prior to Krogh other investigators had observed that the capillaries can be contracted. This is correct, and Krogh himself stressed this fact as he gradually became acquainted with the older literature. Furthermore, contemporary scientists were studying the capillaries—Steinach, Richards, Dale, Ebbecke—but none of them had visualized so clearly the problem as a whole nor understood its importance for the circulation and a large number of other physiological processes. For many years, Krogh therefore directed almost all his strength and the working capacity of his collaborators toward the study of the capillaries.

Even before Krogh had been awarded the Nobel prize, foreign scientists had found their way to his laboratory in Copenhagen. As early as 1915 the first one, the Swedish physiologist G. Liljestrand, had come in order

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[21] Krogh, August and Lindhard, Johannes, with the collaboration of Liljestrand, Göran and Gad-Andresen, Knud: The relative value of fat and carbohydrate as sources of muscular energy. With appendices on the correlation between standard metabolism and the respiratory quotient during rest and work. Biochem. J., Lond., 1920, *14*, 290.

[22] Krogh, August and Lindhard, Johannes: The changes in respiration at the transition from work to rest. J. Physiol., Lond., 1919-20, *53*, 431.

[23] *Op. cit.* (see note 18).

[24] The supply of oxygen to the tissues and the regulation of the capillary circulation. J. Physiol., Lond., 1918-19, *52*, 456.

[25] Studies on the capillariomotor mechanism. I. The reaction to stimuli and the innervation of the blood vessels in the tongue of the frog. J. Physiol., Lond., 1919-20, *53*, 399.

to participate in the investigations of the physiology of exercise—but now scholars arrived from all over the world, especially from the United States of America. There was no lack of problems. Was the observed regulation of the number of open capillaries in the tissue a chemical, a hormonal, or a nervous one? In what way could the capillaries with their mono-layer epithelial walls contract? The young Danish physician, Bj. Vimtrup, was able to show that the frog's capillaries are provided with special muscle cells, the so-called Rouget cells, which can contract the capillaries.[26] Many years later, Beecher, an American co-worker, demonstrated that an epithelial mechanism is also present in mammals, and that, as it was emphasized by other investigators, the capillaries can be closed by swelling of the cell nuclei.[27]

Krogh and his co-workers demonstrated that the opening and closing of the capillaries is effected both by nerves and hormones. As early as 1922, Krogh was able to publish a review of his own studies and the results of other investigators on capillary physiology.[28] This was not his first monograph. In 1916 he had been invited to write a monograph on *The respiratory exchange of animals and man*.[29] Due to its exhaustive treatment of the subject it was immediately acknowledged as the classical textbook in this field. In his new monograph on capillaries we find the same comprehension of his own observations and careful treatment of other scientists' results. These two books are, however, very different. We cannot help noticing that *The anatomy and physiology of capillaries* was written much more freely, much more *con amore*. On almost every page new problems and viewpoints are presented. The respiration monograph is a handbook in which knowledge of that time is compiled and digested; the monograph on capillaries, on the other hand, can be read like a novel. No wonder, therefore, that this book has inspired numerous investigators. It appeared in three editions, and still more editions could have appeared if Krogh had not proceeded to new tasks and felt that his contact with the current literature in this field was no longer sufficient to justify the responsibility for a new issue. Few books have exerted their influence within such varied fields. Drinker's lymph investigations, Mygind's investigations into Ménière's disease, the present author's kidney work—just to mention a few of them—have been inspired in one way or another by Krogh's capillary

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[26] Vimtrup, Bj.: Beiträge zur Anatomie der Capillaren. I. Über contractile Elemente in der Gefäszwand der Blutcapillaren. Zeitschrift für Anatomie und Entwicklungsgeschichte, 1922, *65*, 150; II. Beiträge zur Anatomie der Capillaren Weitere Untersuchungen über contractile Elemente in der Gefäszwand der Blutcapillaren. *Ibid.*, 1923, *68*, 469.

[27] Beecher, H. K.: The independent control of the capillary circulation in a mammal. Skand. Arch. Phys., 1936, *73*, 1.

[28] *The anatomy and physiology of capillaries*. New Haven, Yale University Press, 1922, 276 pp.

[29] *The respiratory exchange of animals and man*. London, Longmans, Green, 1916, 173 pp.

studies. Moreover, scarcely any medical man in research work can escape the influence of Krogh's viewpoints.

Krogh wrote his papers and books in the English language which he mastered to perfection. When I was once asked to find a typical example of modern scientific English, I consulted a British scientist. "Take one of Krogh's chapters," was his answer. Krogh published but very few papers in Danish, and sometimes he was criticized for it; a Danish scientist should write in his native language, he was told. Krogh answered that one serves both one's country and science better by writing in a way suited to make one's works known in wider circles.

Krogh gained the attention of the scientific world through *The anatomy and physiology of capillaries,* and we can plainly speak of a Krogh school in the U.S.A. In order to illustrate Krogh's influence on American physiology it may be mentioned that eight professors on the present staff of Harvard University—among them Drinker, Landis, Churchill, and Beecher —studied with Krogh. The gratitude of American physiologists to Krogh was also manifested by their wish to deliver, in addition to the President's commemorative oration, a special American homage in tribute to Krogh at the recent Physiological Congress in Copenhagen.

In the years immediately after 1920, the capillary studies were continued, especially by investigating the formation of edema[80] and the innervation of the capillaries.[81] Simultaneously, however, Krogh started out on numerous new tasks. During his stay in the U.S.A. in 1922, where he delivered the Silliman lectures on his work on capillaries,[82] Krogh took the opportunity of acquainting himself with the preparation of newly discovered insulin. On his return to Denmark he organized, in collaboration with Hagedorn, the Danish production of insulin. For many years Krogh had been in contact with *Løvens kemiske Fabrikker.* He succeeded in convincing the proprietor that insulin should be manufactured without profit to private persons. The result was the foundation of two institutions, Nordisk Insulin Laboratory and Nordisk Insulin Fond, for many years presided over by Krogh. Both institutions are of great importance for Scandinavian science. Moreover, it has been possible to produce insulin at a much lower price than elsewhere. Krogh also studied the scientific problems involved in insulin treatment, such as its influence upon respiratory metabolism.[83] His main

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[80] Krogh, August and Harrop, G. A.: Quelques remarques sur les stases et les oedèmes. C. rend. Soc. biol., 1921, *84,* 325.

[81] Krogh, August, Harrop, G. A., and Rehberg, P. B.: Studies on the physiology of capillaries. III. The innervation of the blood vessels in the hind legs of the frog. J. Physiol., Lond., 1922, *56,* 179.

[82] *Op. cit.* (see note 28).

[83] Krogh, August and Rehberg, P. B.: The influence of insulin on metabolic processes. Page 91 in volume in honour of I. P. Pavlov's 75th birthday, Leningrad, 1925.

interest, however, he devoted together with Hemmingsen to the problem of the standardization of insulin.[84]

A few years later the Rockefeller Foundation announced their willingness to help improve Krogh's working conditions. At that time, the old laboratory was crowded with guests from abroad. Thus, at one time, C. K. Drinker, A. N. Richards, E. D. Churchill, and several others were at the laboratory; many guests had to be refused. In the course of negotiations with the Rockefeller Foundation an arrangement was made according to which not only Krogh's institute, but five different laboratories were placed in the new stately laboratory building at Juliane Maries Vej. Krogh put his heart and soul into the building of the new institute. Every single room was drawn with all its details and a plan was made for every single wall. He surveyed the trees in the neighborhood and calculated their shadows at various seasons of the year. Many new improvements were devised, and the building, which was ready at the end of 1928, is largely stamped with Krogh's effort and differs in many respects from other institutes. Naturally this applies mainly to Krogh's own department which during the following years—and, by the way, even today—is visited by many people who are about to build their own institute. As an example of an unusual idea of Krogh's it may be mentioned that he made an attempt at building especially dust-free rooms and store rooms in the laboratory. He was not completely successful because of poor materials, but later this ideal was achieved, for example, in the new University Library. It is a specialty of the Zoophysiological Laboratory (the new name of the department) that in all rooms the furnishings and equipment can easily be moved and re-collected in another way.

Scholars from all over the world came to work in the new laboratory, and many young Danish scientists were informally appointed to the staff. In the first years, the investigations of the physiology of exercise mentioned above were performed, and Krogh continued to work out new methods—for example, one for the determination of the total osmotic pressure of the blood.[85] Moreover, he constructed a marvellous balance which is able to register the weight of a human with an accuracy of *ca.* 2 gm., even while the test person rides a bicycle ergometer suspended on the balance.[86]

There is one more problem in the field of human physiology which Krogh succeeded in treating, namely, heat regulation in man, especially the physi-

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[84] Krogh, August and Hemmingsen, A. M.: The assay of insulin by the convulsive-dose method on white mice. Publications of the League of Nations III. Health, 1926, III, *7*, 40.

[85] Covián, Fr. Grande and Krogh, August: The change in osmotic pressure and total concentration of the blood in man during and after muscular work. Skand. Arch. Physiol., 1935, *71*, 251.

[86] Krogh, August and Trolle, Carsten: A balance for the determination of insensible perspiration in man and its use. Skand. Arch. Physiol., 1936, *73*, 159.

ological problems of heating houses. His activity in this field was concentrated in the House Heating Commission which, shortly before the war, was established by the Academy for the Technical Sciences with Krogh as president. He took an active part in the planning of both the technical and the scientific investigations. Similarly, in this case his ingenious ability as a constructor of apparatus was of decisive influence for experimental methodology. In this connection it is worth mentioning his microclimatograph which consists of a wrist watch with the clockwork reconstructed so that it can register temperature and humidity on a smoked glass plate.[37] The apparatus has proved useful in many other investigations and is especially applicable to ecological studies. The physiological part of the investigations was entrusted to Marius Nielsen and Lorentz Pedersen, the physiological results being published together with the technical ones in an extensive series of publications in 1947-48;[38] and even after this time Krogh was interested in these problems and new experiments were started shortly before his death.

As it appears from this review of Krogh's work on human physiology, his research studies covered a large number of fields—respiration, metabolism, circulation, insulin, and heat regulation—but he made still further excursions to other domains, though abandoning them rapidly. It is through the above mentioned work that he gained his great name and fame among scientists. And yet this is but one side of his scientific activity.

His achievements as a zoophysiologist are equally extensive and significant, and his work within this realm was continued throughout the years, from his first papers on hydrostatic mechanics[39] to the last ones on the production of heat in insects.[40] Krogh never lost his interest in zoophysiology—on the contrary, this was perhaps what attracted him most. Thus, in a speech to the Congress in Boston in 1929 he stated: "You will find in the lower animals mechanisms and adaptations of exquisite beauty and the most surprising character, and I think nothing can be more fascinating than the senses and instincts of insects as revealed by the modern investigations."[41] Thus he actually refrained from work on zoophysiological problems only at times when practical work forced him to do so, for example during his insulin period; otherwise one could be quite sure that he was engaged in one or another zoophysiological study.

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[37] A micro-climate recorder. Ecology, 1940, *21*, 275.

[38] Boligopvarmningsudvalgets Meddelelser: Nos. 2, 3, 1947 and Nos. 4, 5, 6, 7, 9, 10, 11, 1948.

[39] Om Turgescens Betydning for Plantelegmets Fasthed og Spalteaabningernes Mekanik. Biol. Selsk. Forh. i Vinterhalvaaret, 1900-1901, p. 4; Om hydrostatiske Forhold i Dyreriget, *Ibid.*, p. 14.

[40] Determination of temperature and heat production in insects. Zeitschr. vergl. Physiol., 1948, *31*, 274.

[41] Krogh, August: The progress of physiology. Science, 1929, *70*, 203; also in Am. J. Physiol., 1929, *90*, 247.

Also in his zoophysiological work a definite trend can be followed through the years. As mentioned before, when a student he started on his investigations into the hydrostatic mechanism of *Corethra* larvae, and this problem was resumed many years later. Likewise the problem of the respiration of insects was taken up again, applying the improved methods of air analysis.[42] His micro air analysis apparatus, designed for the investigation of gas tension in blood, had opened the possibility of studying the respiration of the trachea, an investigation which had not been feasible before.[43] Krogh demonstrated the great significance of diffusion for the respiration of insects and its rôle as a restricting factor. Somewhat later he constructed his micro-respiration apparatus which makes a determination of the metabolism of single insects possible. Krogh used the method to investigate the influence of temperature on the metabolism of insects. This work led him to similar studies in other animals and, furthermore, to the discovery that the metabolism increases with temperature according to a Van't Hoff curve. The influence of temperature on metabolism and respiration is dealt with in *The respiratory exchange of animals and man*,[44] which contains a multitude of material from the fields of comparative physiology. Inserted among the other papers appears an investigation, in collaboration with Schmit-Jensen, on the cellulose fermentation in the paunch of ruminants[45] and, in addition, one, in collaboration with I. Leitch, on the hemoglobin of fishes,[46] which was shown to be adapted to the oxygen tension of the environment.

After the strenuous period of work on the capillaries and insulin and the ensuing intermission in his zoophysiological activities, Krogh turned to the problem of metabolism in the sea. Is it possible, as stated by Pütter, that certain aquatic animals can live on dissolved material? For a number of years Krogh, in collaboration with Spärck, conducted studies on the biology of oysters.[47] This series of investigations, the results of which have

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[42] *The comparative physiology of respiratory mechanisms*. Philadelphia, The University of Pennsylvania Press, 1941, vii, 172 pp.

[43] On the composition of the air in the tracheal system of some insects. Skand. Arch. Physiol., 1913, *29*, 29.

Studien über Tracheenrespiration II. Ueber Gasdiffusion in den Tracheen. Pflügers Arch., 1920, *179*, 95.

Studien über Tracheenrespiration III. Die Kombination von mechanischen Ventilation mit Gasdiffusion nach Versuchen an Dytiscuslarven. Pflügers Arch., 1920, *179*, 113.

[44] See note 29.

[45] Krogh, August and Schmit-Jensen, H. O.: The fermentation of cellulose in the paunch of the ox and its significance in metabolism experiments. Biochem. J., Lond., 1920, *14*, 686.

[46] Krogh, August and Leitch, I. The respiratory function of the blood in fishes. J. Physiol., Lond., 1919, *52*, 288.

[47] Gaarder, Torbjørn and Spärck, R.: Biochemical and biological investigations of the variations in the productivity of the West Norwegian oyster pools. Conseil pour l'Exploration de la Mer. Rapports et Proces-Verbaux 1931, *75*, 47; also *Hydrographisch-biochemische Untersuchungen in norwegischen Austern-Pollen*. Bergens Museums Aarbok, Naturvidenskabelig rekke, 1932, No. 1, 144 pp.

been published by other scientists (in the first place by Gaarder and Spärck), was performed in a West Norwegian pond rented by the laboratory; here, Norwegian and Danish scientists investigated the conditions for the propagation of oysters. Krogh took a lively part in the discussions and planning of these experiments which lasted many years and were very costly; but despite their high expense the costs of these experiments were covered by the sale of oyster breedings. On the whole, Krogh was always successful in conjuring the funds required for the intensive work at the laboratory, to which the State contributed only a very modest sum. The remaining part was raised from outside funds or in some other way, for example by running the laboratory workshop on a purely commercial basis. All apparatus designed in the laboratory, and this means primarily by Krogh himself, was built in the workshop and sold without profit for the designer, the total income being the laboratory's. Thus, the basal metabolism apparatus constructed by Krogh on the basis of the "tilting spirometer" was sold by the hundreds to hospitals and doctors. The income of the laboratory in the course of years amounted to a figure of six digits.

The investigations on metabolism in the sea, which had now started, required new methods. Again Krogh was the man to develop them: methods for the quantitative determination of the chemical composition of the plankton[48] as well as for the study of the quantity of dissolved nutrient substances in water.[49]

It may be noted that at a much earlier date, Krogh had been interested in oceanographic investigations. During his first stay in Greenland (1902), he measured carbon dioxide in sea water[50] and established our knowledge of the equilibrium between the carbon dioxide content of the air and sea water,[51] which is so important as a regulating factor for the carbon dioxide content of the air. With the help of his precision air analysis apparatus Krogh also analyzed the composition of the atmosphere and he stated the extreme constancy of the latter.[52] At the same time he demonstrated how small the changes were even in a city of the size of Copenhagen.[53]

In connection with the work on the diet of aquatic animals, which finally refuted Pütter's theory of the significance of dissolved substances, Krogh started working on the problem of the osmoregulation of aquatic animals. To these studies, where investigations into the permeability of the skin

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[48] Eine Mikromethode für die organische Verbrennungs-analyse, besonders von gelösten Substanzen. Biochem. Zschr., 1930, *221*, 247.

[49] Krogh, August and Keys, Ancel: Methods for the determination of dissolved organic carbon and nitrogen in sea water. Biol. Bull., 1934, *67*, 132.

[50] On the tension of carbonic acid in natural waters and especially in the sea. Medd. om Grønland, 1904, *26*, 331.

[51] The abnormal CO_2 percentage in the air in Greenland and the general relations between atmospheric and oceanic carbonic acid. *Ibid.*, 1904, *26*, 407.

[52] The composition of the atmosphere. An account of preliminary investigations and a programme. K.D. Vid. Selsk. Fys. Medd., 1919, *I*, 12.

[53] See note 52.

play an important part, Krogh applied as early as 1935 the nowadays extensively used isotopes, stressing their enormous applicability in biology.[54] In the years to come the osmoregulatory studies were continued and, in 1939, Krogh summarized our knowledge in this sphere in a monograph on *Osmotic regulation in aquatic animals*[55] which, like the lectures on capillaries, immediately became the classic book in this field and a source from which many investigators have received inspiration.

About a year later Krogh accomplished the publication of another monograph on *The comparative physiology of respiratory mechanisms*,[56] reviewing all the knowledge gained in this domain in the course of the twenty-five years which had elapsed since the publication of his first monograph on almost the same subject.[57] This book and the viewpoints expressed in it will certainly become a gold-mine for zoophysiologists.

Again and again Krogh resumed problems which had caught his attention during one period or the other. Since his very youth he had been interested in the flight of birds and insects; throughout the years he repeatedly discussed the possibilities of studying more thoroughly the mechanism and energy balance of animal flight, examining the various ways of attacking these problems. As late as 1945, after his retirement from the professorship and after having moved to his private laboratory, he found opportunity for resuming study of this question. Again his imagination and his ability as an experimenter were unique. Investigations on the flight of grasshoppers were performed partly by means of a grasshopper merry-go-round carrying 32 grasshoppers, partly with single animals in an air tunnel. When published by his co-worker Weis-Fogh, these studies will undoubtedly prove to be basic for our knowledge in this domain.

As already mentioned, Krogh's very first work was a contribution to the sap tension in plants.[58] In his later years he came back to related problems investigating, in collaboration with the Swedish plant physiologist, Hans Burström, the flow of the sap of trees in relation to the development of the buds.[59] These investigations were performed during his forced emigration towards the end of the war, when it was found safest for him to settle in Sweden.

Krogh's scientific production embraces numerous fields, many more than are mentioned here, and almost three hundred papers are included in his bibliography. Here I wish to refer to the lists of his publications found both in the Communications of the Academy for the Technical Sci-

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[54] The use of isotopes in biological research. Orvosi Hétilap, Budapest, 1935.
[55] Krogh, August: *Osmotic regulation in aquatic animals.* Cambridge, Cambridge University Press, 1939, 242 pp.
[56] *Op. cit.* (see note 42).
[57] *Op. cit.* (see note 29).
[58] *Op. cit.* (see note 39).
[59] Krogh, August and Burström, Hans: The biochemistry of the development of buds in trees and the bleeding sap. K. D. Vid. Selsk., Biol. Medd., 1946, *20*, 2.

ences,[80] and in the *Vidensk. Medd. fra Dansk naturhistorisk Forening, København.*[81] Krogh's significance in science is not limited to his scientific production. He has been an important guide for other scientists, mostly Danes, but also numerous foreigners who came for his advice concerning problems which frequently were outside his scientific domain. But even if a subject was ever so remote from his thoughts, Krogh was always ready to listen to his visitor; as soon as his interest was caught he was able to give advice and the interviewer did well to follow his suggestions. If, on the other hand, a young scientist approached him to have a problem suggested, he came in vain.

For many years it was easy to come and talk with Krogh on scientific matters; nobody was refused an interview. In later years, however, Krogh found it necessary to economize his time—so many things were left for him to do—and therefore he asked visitors to look for advice elsewhere. But if one was admitted, an "interview" with him took a very typical course. First, Krogh asked the visitor to talk about his problem. In the beginning he sat listening with his head somewhat bowed to the side and a scrutinizing glance in his eyes. Then he asked a single question, more questions, and somewhat later, maybe some hours later, he would start speaking, and if his interest had actually been awakened he was generous with his time. He then gave his advice, whether to continue or to stop, whether there was a practicable way to go, and where it led. Not all who came understood that it was a good thing to follow his advice; maybe some actually did not want to learn, but would rather have had their own ideas confirmed, while others may have assumed that Krogh did not understand their problem as profoundly as they did themselves. Therefore they went away angry because he had not approved of their ideas. Many of them certainly regretted that they had not listened to him and relinquished a plan. Krogh's intuitive judgment of whether a goal could be reached in a given way was fabulous even in fields remote from his thoughts. This capability was also of enormous value to his own work. Beyond doubt, there are few scientists who initiated so many diverse problems and yet were able successfully to complete so many.

Also, for young scientists at the laboratory Krogh was an outstanding teacher not only of the experimental procedure to attack a problem, but, of course, primarily in making them comprehend the interrelationship of the problems as a whole. Nobody who worked at his side for even a short time could help being influenced by his way of working. Krogh was opposed to the simple collecting of scientific facts: "Facts are necessary, of course, but unless fertilized by ideas, correlated with other facts, illuminated by

[80] Fortegnelse over August Krogh's publikationer, revideret af dr. phil. Axel M. Hemmingensen. Meddelelser fra Akademiet for de tekniske videnskaber., 1949, No. *1*, 39.

[81] *Op. cit.* (see note 17).

thought, I consider them as material only for science . . . Too many experiments and observations are being made and published and too little thought is bestowed upon them," he once said in a speech,[62] and it was this doctrine he taught the young generation.

On the other hand, Krogh was always very reluctant to encourage a young man to become a scientist. He did not advocate such decisions, he rather warned against such a career. It was his view that the requirements for a degree in zoophysiology should be such that only the highly interested and capable candidates could pass. He had the great pleasure to see his children and children-in-law develop notable scientific abilities. Not many scientists receive on their seventieth anniversary a volume dedicated to them which contains seven scientific papers written by four children and three sons-in-law and with a preface by a daughter-in-law.[63]

On his fiftieth birthday his pupils published a fine book in his honour —a tribute paid as a rule to a scientist of a more advanced age.[64] If they had waited until his sixtieth birthday, an edition of one volume would not have been sufficient.

Krogh's lectures were brilliant, perhaps not so much for their form as for their content. He made great effort to be up to date in the whole field, and all new discoveries and viewpoints were included in his lectures. It was intellectually gratifying to attend his examinations, but for many candidates they certainly were too difficult; it was hard for many of them to grasp the elementary aspects. On the other hand, Krogh was an unsurpassed teacher for advanced students and hardly any one of them left his lectures without realizing that he had been in touch with an outstanding scientist.

As the years went by, Krogh became tired of the great burden of preparing the lectures and, from 1934, he was given dispensation from his teaching load. He then gave only a few series of lectures on special topics (osmoregulation, comparative respiratory physiology, etc.) From then on, he had little contact with the students, most of whom knew him only as the author of the well-known textbook.[65] This book was the result of another of Krogh's activities. When in 1903 new regulations were issued for high school teaching, it was decided that the treatment of man in the natural history classes should be based on physiological rather than anatomi-

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[62] Krogh, August: The progress of physiology. Science, 1929, *70*, 203; also in Am. J. Physiol., 1929, *90*, 248.

[63] Krogh, August. Dr. phil., Dr. jur., Dr. med., Dr. sc. Til hans 70 Aars Fødselsdag 15 November 1944 (Udg. af August Kroghs Børn og Svigerbørn). (Red. af Knut Schmidt-Nielsen) 76S. ill. og 3 Tvlr. 4°. 44. Privattryk. Ikke i Bogh.

[64] *Physiological papers, dedicated to Prof. August Krogh.* R. Ege, H. C. Hagedorn, *et al.*, Eds. Copenhagen, Levin and Munksgaard, 1926; London, Wm. Heinemann Ltd., 1926. xvi, 375 pp.

[65] *Kortfattet Lærebog i menneskets Fysiologi (for Gymnasiets mat.—naturv. Linie).* København, Gyldendal, First ed., 1908; Second-tenth eds., 1912-1946. Ninth and tenth eds. in collaboration with Prof. P. Brandt Rehberg. 130 pp.

cal views. In 1908, Krogh's *Text book on the physiology of man* was published for the first time. In its way this book is just as excellent as Krogh's scientific publications. In about a hundred pages the physiology of man is reviewed in such a way as to be read to everybody's profit by people of widely varying backgrounds. The more one knows to start with, the more one finds in this book. It is both easy and extremely difficult, because it contains so much information for a reader who tries to penetrate the depths of the sentences. For forty years this book has been the only Danish school book on this subject and it has appeared in eleven editions. Although the character of the book is preserved and the contents changed only slightly, every new edition meant hard work, and by replacing a few words he could frequently express quite new viewpoints. Undoubtedly because of this book, general knowledge of physiology for many years was greater among students in Denmark than in other countries, although by now the difference may have disappeared.

Krogh felt closely attached to high school teachers and he understood the significance of keeping the teachers *à jour* with the progress in physiology. He achieved this partly by lecturing and partly by publishing papers in the pōpulār periodical *Naturens Verden*. In these articles he often went outside his own field and dealt, for example, with brain physiology, especially with Pavlov's conditioned reflexes, and with sense physiology—two domains which he never entered in his own research but in which he always was vitally interested. On the whole, his biological interests were very wide; thus, for example, he was an expert on Danish flora. Moreover, his old love of the sea induced him to spend a lot of time during the summer vacations in a small jolly-boat on the Isefjord, studying animal life.

Krogh was extremely industrious. He started work in his laboratory early in the morning and the cleaning personnel were not allowed to enter his rooms before 9 o'clock. From 9 to 10 o'clock he paused, then he continued and as a rule did not stop before late in the evening. Once when an interviewer asked about his hobby, he answered, "My work."

Despite this enormous diligence which almost grew in the course of time, he nevertheless had the leisure to take an interest in the problems of the day. He read a great deal, preferably British and American literature. I remember that his library at the time I first met him included a large number of books dealing with naval history and the complete works of Kipling and other British authors. He knew his Kipling so well that he was able to send quoted passages with the statement of the first use of their words to the *Oxford Dictionary*. He liked to read aloud from Kipling, and many of his friends keep a pleasant remembrance of a party of children and friends gathered in front of the fireplace while one of Kipling's stories was read for them. Krogh was also vividly interested in history and archaeology and, not least, in the history of science and its

development. In collaboration with V. Maar he translated and published Steno's *Preliminary communication of a treatise on solid bodies which are naturally embedded in other solid bodies.*[66] He has written repeatedly about Steno, and his very last literary work is an essay on Steno in the book *Prominent Danish personalities.*[67]

A peculiar feature of his mind was his attitude towards music. Not only was he lacking in an ear for music—he simply disliked music, which he considered a kind of disturbing noise causing him almost physical discomfort.

Krogh's attitude towards other people was frequently determined by a first impression or by one or another reaction of the person in question. From this impression Krogh formed his picture. This might appear arbitrary and subjective, but as a rule he turned out to be right even though he himself would not deny that he possibly could be unjust. To him things were either black or white. If he was your friend, he was it completely and usually forever; was he your enemy, he very rarely changed his mind. If he had reason for mistrust he was through with the man. To him applied William Sørensen's motto, "In the last instance everything depends on the character." Something of this attitude was also to be found in his scientific criticism. He could be violent in scientific controversies, which he considered almost useful for science, since they appeared to him to be a necessary link in the comprehension of truth. But, in a speech given in Boston in 1929, he says apologetically: "We may fondly imagine that we are impartial seekers after truth, but with a few exceptions, to which I know that I do not belong, we are influenced and sometimes strongly by our personal bias and we give our best thoughts to those ideas which we have to defend."[68]

It was the same uncompromising attitude which resulted in his passionately discussed resignation from the Royal Danish Academy of which he had been a member since 1916. He had given numerous lectures in the Academy and he had been a keen participant in the sessions for many years. The reason for his resignation was that he failed to induce the Academy to take a more active attitude concerning the economic support of science, and he thought that in the long run his action would prove useful. "At least it has shown the authorities that if they want to do something positive public opinion will be behind them," he wrote in a letter to an American friend.

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[66] Steno, Nicolaus: *Foreløbig meddelelse til en afhandling Om faste legemer, der findes naturlig indlejrede i andre faste legemer.* I oversaettelse ved August Krogh og Vilhelm Maar. Med indledning og noter. København, Gyldendal, 1902, 106 pp.

[67] Niels Steensen 1638-1686. Store danske Personligheder, Berlingske Forlag, Kbhvn., 1949, p. 44.

[68] The progress of physiology. Science, 1929, *70*, 203; also in Am. J. Physiol., 1929, *90*, 248.

Krogh did not attach much importance to formal honours and refused to accept decorations. On the other hand, he was very appreciative of tributes of respect from his colleagues, for example, membership of numerous foreign societies and the six honorary degrees which he was awarded in the course of time.

His affiliation with Copenhagen University meant very much to him. Admittedly, he was not interested in sharing the University's administrative duties; according to his own view, he had only two obligations: to teach and to do research, the latter above all. Therefore he refused the deanship, nevertheless taking an active part in discussions of the faculty concerning the teaching program or the problems of promoting scientific work; in these fields his opinion frequently was of decisive influence.

Danish science has suffered a great loss in the death of August Krogh. His working ability was practically unimpaired until his final illness, and until his last day he was vividly interested in a great variety of problems.[60] If he had been allowed to live, many new discoveries would certainly have come to light.

[60] His last paper, which was actually published after his death, was: Reminiscences of work on capillary circulation. Isis, 1950, *41*, 14.

THE INFLUENCE OF DIET AND INSULIN ON THE INCIDENCE OF CATARACTS IN DIABETIC RATS*

RICARDO R. RODRIGUEZ† AND WILLARD A. KREHL

The appearance of cataracts in animals with experimental diabetes has been described in dogs with pituitary[6] or pancreatic diabetes,[2, 3] in rats with pancreatic,[5, 9] alloxan[1, 4, 11] or dehydro-ascorbic acid diabetes,[12] and in alloxan diabetic rabbits.[1, 7] According to some of these investigators[4, 12, 11] the appearance of cataracts is correlated with the degree of hyperglycemia and glycosuria. The observations reported in this paper on partially pancreatectomized and alloxan-diabetic rats demonstrate further the influence of diet and the level of circulating blood glucose on the development of cataracts, and the importance of insulin in the control of the diabetic state and the consequent prevention of cataract development.

MATERIALS AND METHODS

Male rats were used throughout the investigation. In the first experiment upon rats of the Yale strain of 80 to 100 g. of body weight, 95% of the pancreas was removed using Foglia's technique.[8] In the second experiment, rats of the Wistar strain weighing between 100 and 150 g. were made diabetic by the intraperitoneal injection of alloxan, 10 mg./kg.

All animals in both experiments were given food and water *ad libitum* and were weighed at least once a week. The diets used were of the "synthetic" type; their composition is shown in Table 1. It will be noted that all of the diets were iso-caloric so that animals on the high fat diets were receiving approximately the same percentage of protein calories and vitamins as animals on the high carbohydrate diet. Food intake on the various diets was determined at routine intervals in order to compare and relate the gains in weight to caloric ingestion in both diabetic and non-diabetic rats.

Each rat was housed in an individual screen bottom cage in an air-conditioned room held at a temperature of 25-26° C. with a relative humidity of 50-55 per cent.

Urinary glucose was determined in the fasting state at monthly intervals in the partially pancreatectomized animals, and ten days after alloxan administration in the injected rats in order to ascertain the number of animals that were diabetic. After the incidence of diabetes had been determined, non-fasting glycosuria was estimated at monthly intervals to establish the degree of diabetes and the amount of glucose excreted in the urine. In all cases, urine was collected under toluol, and Benedict's quantitative method was used for the determination of glucose in the sample.

The eyes of the rats were examined once a week, and the time at which the lens became opaque was noted.

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Results

The data in Table 2 show that in partially pancreatectomized rats the type of diet exerts an influence on the incidence of diabetes. In agreement with the results of Houssay and Martinez,[10] the high protein diet markedly reduced the incidence of diabetes, (at six months, 80% were diabetic on the high carbohydrate diet, but only 33% on the high protein diet). The incidence of diabetes (83%) after six months on the high fat diet* was almost

Table 1

Composition of Synthetic (Iso-Caloric) Diets

	High carbohydrate	*High protein*	*High fat*	*High carbohydrate plus cholesterol*	*High carbohydrate minus choline*
"Labco" casein	15	55	15	15	15
Vitaminized casein*	5	5	5	5	5
Vitaminized corn oil†	5	5	5	5	5
Lard ("Swift'ning")	5	5	34.2	5	5
Dextrin	65.7	25.7	...	65.2	65.8
Salts IV	4	4	4	4	4
Cholesterol	...	...	...	0.5	...
Cystine	0.2	0.2	0.2	0.2	0.2
Choline	0.1	0.1	0.1	0.1	0.0
	100.0	100.0	100.0	100.0	100.0

* Supplies adequate levels of B-complex vitamins and Menadione.
† Contains added fat-soluble vitamins A, D, and E.

the same as on the high carbohydrate diet (80%). The incidence of diabetes after six months on the high carbohydrate diet either with 0.5 per cent cholesterol added, or without added choline, was 65% and 66%, respectively.

That the incidence of cataracts in the diabetic state is the resultant of the combined effects of type of diet and degree of glycosuria (a reflection of glycemia) is seen from an examination of the data in Tables 3 and 4. On the same diet, both the incidence of cataracts and the glycosuria were higher in alloxan diabetes than in the animals made diabetic by partial pancreatectomy. On the high carbohydrate diet, where the glycosuria was highest, the maximum incidence of cataracts was observed. On the other hand, comparable animals on the same diet with their diabetes controlled with insulin so that the glycosuria was held to less than one gram per rat

* The fat used in these experiments (Swift'ning) is a mixture of vegetable and animal fats. The incidence of pancreatic and alloxan diabetes is higher in rats fed on a high fat (lard) diet, and lower in rats fed on a high fat (coconut oil) diet.

per day showed absolutely no evidence of cataract formation. This complete protective action of insulin has now been extended to ten months.

No cataract was observed in the partially pancreatectomized animals on the high protein diet, while alloxan diabetic animals on the same diet

TABLE 2

INFLUENCE OF DIET ON THE INCIDENCE OF DIABETES IN 95% PARTIALLY PANCREATECTOMIZED RATS

*Months after Ppx,*95%*	*High carbohydrate*		*High fat*		*High protein*		*High carbohydrate plus cholesterol*		*High carbohydrate minus choline*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1	17/31	55	5/24	21	10/24	41	10/23	43	10/24	41
2	23/31	74	19/24	79	7/23	30	18/23	78	14/24	58
3	24/31	77	19/24	79	8/24	33	17/23	73	18/24	75
4	24/31	77	20/24	83	9/24	37	16/23	69	17/24	71
5	25/31	80	20/24	83	8/24	33	16/23	69	15/24	62
6	25/31	80	20/24	83	8/24	33	15/23	65	16/24	66

* Ppx = partial pancreatectomy.

TABLE 3

INFLUENCE OF DIET ON THE INCIDENCE OF CATARACTS IN RATS AFTER SIX MONTHS OF DIABETES

	High carbohydrate									
	Without insulin		*With insulin*		*High protein*		*High fat*		*High carbohydrate plus cholesterol*		*High carbohydrate minus choline*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Pancreatic diabetes	5/13	38	0/12	0	0/8	0	0/20	0	2/11	18	2/11	18
Alloxan diabetes	20/25	80	....	..	1/5	20	0/20	0	....	..	....	..

exhibited an incidence of cataracts of 20%. It will be noted in Table 4 that the glycosuria in the pancreatectomized animals on the high protein diet was less than one gram per rat per day, while the alloxan diabetic animals were excreting over two grams of glucose daily. No cataract was observed in any animal receiving the high fat diet, and all animals on this diet excreted in their urine less than 0.5 grams of glucose per day (Table 4).

None of the control rats (partially pancreatectomized and alloxan injected, non-diabetic animals) developed cataracts during the six months of the experiment.

A few (less than 10%) of the diabetic rats on the high carbohydrate diet, with pronounced glycosuria (nearly seven grams per rat per day), did not develop cataracts during the six months of observation.

Discussion

From the results reported here and elsewhere, the development of cataracts in diabetic rats is seen to be influenced by at least three factors: (a) age of the animals, (b) degree of hyperglycemia and glycosuria, and (c) duration of diabetes.

Age is an important factor in the development of cataracts since cataracts appear earlier in young than in old rats. In one earlier exploratory experiment, pregnant rats were made diabetic with alloxan on the 12th day of pregnancy. At the end of the gestation period some of the rats with severe diabetes delivered young which were given to non-diabetic mothers to nurse. These newborn rats from diabetic mothers had normal blood glucose levels and normal glucose tolerance curves and at no time exhibited symptoms of diabetes. It was observed, however, that about 50% of the young developed cataracts, visible in both eyes when they opened their eyes for the first time, at approximately 15 days of age.[18] From this result, it would appear that in the earlier stages of lens development, only nine days of hyperglycemia are necessary in order to produce cataracts.

Table 4

Urinary Excretion of Glucose in Alloxan and Partially Pancreatomized Diabetic Rats

	Glycosuria (g./rat/day)					
	High carbohydrate					
Diet	*Without insulin*	*With insulin*	*High protein*	*High fat*	*High carbohydrate plus cholesterol*	*High carbohydrate minus choline*
Alloxan diabetes	5.13	...	2.27	.27	...	...
Pancreatic diabetes	2.41	.72	.85	.37	2.44	2.12

All of the evidence obtained in these experiments points to a direct correlation between the intensity of hyperglycemia and glycosuria, the duration of diabetes, and the onset of cataracts. The higher the glycosuria, the shorter is the period of time before the development of cataracts.

Further support of the idea that the level of blood glucose is an etiological factor in the development of cataract is seen from the fact that although rats on the high fat diet had as high an incidence of diabetes as animals on the high carbohydrate diet, their level of glycosuria was less than 0.5 g.

per rat per day and their incidence of cataract was zero. In addition, in every instance where the level of glucose excretion was held to less than one gram per day, whether by giving a high protein diet or by control of the diabetes through the use of insulin, the incidence of cataracts after six months was zero. A comparable, protective action of insulin in a short-term experiment (three weeks) in alloxan diabetes has been reportd by Kok van Alphen and Cohen.[11]

The somewhat lower incidence of both diabetes and cataracts in partially pancreatectomized rats fed the high carbohydrate diet plus cholesterol or minus choline chloride—lower than in those fed the high carbohydrate diet—may be explained by the fact that the daily food intake of the former was less (about 17.7 and 17.8 g. per day compared with 19.5 g. per day).

When cataracts developed in those rats with severe diabetes, both eyes were involved at approximately the same time. On the other hand, in rats with moderate diabetes, especially in the partially pancreatectomized groups, cataracts were sometimes unilateral, with the second eye becoming involved after several weeks.

The influence of these diets and of treatment with insulin on kidney and arteriosclerotic lesions will appear in a future publication.

Summary

The influence of different diets fed during six months on the incidence of diabetes in rats after subtotal pancreatectomy and on the development of cataracts in partially pancreatectomized and alloxan diabetic rats was studied, and the following results were obtained.

The incidence of diabetes decreased in 95% pancreatectomized animals fed a high protein diet, while only a slight reduction was observed in the incidence and severity of diabetes with the addition of cholesterol or the removal of choline in the high carbohydrate diet.

Development of cataracts was higher in diabetic rats fed a high carbohydrate diet; a lesser incidence was observed in high protein fed animals, while no cataracts developed in rats fed a high fat diet.

Treatment with insulin can prevent the onset of cataracts in rats with diabetes of at least ten months' duration.

In summary, the development of diabetic cataracts is directly correlated with the severity of the disease (hyperglycemia and glycosuria) and the duration of diabetes, and inversely correlated with the age of the rats.

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References

1 Bailey, C. C., Bailey, D. T., and Leech, R. S.: Alloxan diabetes with diabetic complications. N. England J. M., 1944, *230*, 533.

2 Chaikoff, I. L. and Lachman, G. S.: Occurrence of cataract in experimental pancreatic diabetes. Proc. Soc. Exp. Biol., N. Y., 1933, *31*, 237.

3 Chaikoff, I. L.: Survival of two depancreatized dogs treated with insulin. Proc. Soc. Exp. Biol., N. Y., 1935, *33*, 211.
4 Charalampous, F. C. and Hegsted, D. M.: Effect of age and diet on development of cataracts in the diabetic rat. Am. J. Physiol., 1950, *161*, 540.
5 Cramer, F. K. and Foglia, V. G.: Catarata diabetica en la rata. Rev. Soc. Argent. Biol., 1943, *19*, 483.
6 Dohan, F., Fish, G., and Lukens, F.: Introduction and course of permanent diabetes produced by anterior pituitary extract. Endocrinology, 1941, *28*, 341.
7 Duffy, E. J.: Alloxan diabetes in the rabbit. J. Path. Bact., Lond., 1945, *57*, 199.
8 Foglia, V. G.: Caracteristicas de la diabetes en la rata. Rev. Soc. argent. biol., 1944, *20*, 21.
9 Foglia, V. G. and Cramer, F. K.: Experimental production of diabetic cataract in the rat. Proc. Soc. Exp. Biol., N. Y., 1944, *55*, 218.
10 Houssay, B. A. and Martinez, C.: Experimental diabetes and diet. Science, 1947, *105*, 548.
11 Kok Van Alpehn, C. C. and Cohen, E. M.: The occurrence of cataract in alloxan diabetic rats. Acta Physiol. Pharm. Neerl., 1950, *1*, 400.
12 Patterson, J. W.: Course of diabetes and development of cataracts after injecting dehydroascorbic acid and related substances. Am. J. Physiol., 1951, *165*, 61.
13 Rodriguez, R. R.: Unpublished observations.

SUSCEPTIBILITY TO FIBROSARCOMAS IN 2NHO MICE*

LEONELL C. STRONG

In the highly inbred Strong A strain of mice, an attempt to produce divergent sub-strains with regard to age distribution of spontaneous mammary adenocarcinomas by means of selective breeding proved ineffectual.[6] It was concluded that this result was an exemplification of Johannsen's genetic principle that pure lines are not amenable to selection.

Conversely, Kreyberg[3] succeeded in selecting divergent strains of mice with regard to susceptibility to tar-induced tumors; his original mice were not from pure lines. Kreyberg did not use age distribution as his criterion, but percentage incidence at comparable ages.

In the establishment of the NHO stock,[6] the object was to obtain many heterozygous gene pairs by strain crossing, so that a broad field for selective breeding would be opened. At the same time, however, a new problem was introduced, namely, heterosis. Although this may not affect susceptibility to tumors directly, heterosis may well affect it indirectly through its effects on disease resistance, reproduction, longevity, and associated physiology. The NHO stock was produced by hybridizing three pure strains, JK, CBA, and N, each different from the others by independent descent. They differ also in several color genes.[6]

The subsequent generations of the NHO stock, inbred brother-sister, and uniformly tested as to tumor induction with methylcholanthrene, have shown some remarkable changes. Selection toward resistance to tumor induction (increased latent period) was started in F_4 and continued to F_{30}. Selection in all sublines (Br, BrS, pBr, Brpb, and pBrpb) was temporarily effective in increasing the latent period for all types of tumors (total number). However, with continuing selection a contrary trend appeared, counter to selection, of lower and lower average latent periods.[7, 10] We have then a paradoxical effect: the final generations of inbreeding show similar resistance to that of the first generations (heterotic).

In this paper data on the effect of selection toward resistance to methylcholanthrene-induced tumors are presented, beginning with the F_1 generation of a new NHO subline and continuing to the F_{20}. This is the period of biological variability by reducing the potential number of heterozygous pairs of genes.[2] Heterosis was also being reduced, since no attempt was made to pick out the healthiest mice in each generation.

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Materials and Methods

The 2NHO strain of mice was established in 1943 by outcrossing mice of the pBr subline (genetic constitution ppaabbSeSe) of the NHO strain to mice of the JK strain (genetic constitution ppaabbsese) (Fig. 1). The 2NHO mice were then continued for twenty generations. Following the elimination of the short ear gene derived from the JK mice in the early hybrid generations, the 2NHO mice had the genetic constitution ppaabbSeSe.

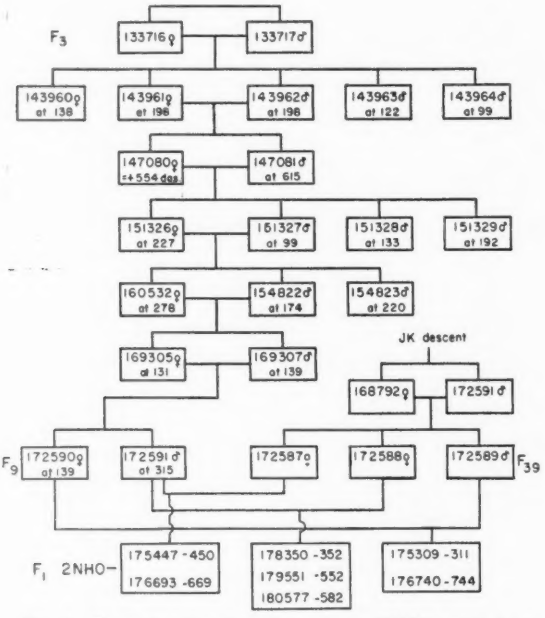

Fig. 1. Chart showing the origin of the 2NHO strain by a cross between the NHO (F_9) and the JK (F_{39}) strains. Latent periods of methycholanthrene-induced fibrosarcomas are given by days, below serial numbers of mice.

During the time the experimental mice were continued, adenomas of the lung were the only tumors which occurred spontaneously in control 2NHO mice, 18 in 188 mice (9.57%) with an average latent period of 776.3 days (747.2 days for females; 836.5 days for males). This is comparable to the incidence of similar tumors in mice of the pBr strain (23 lung tumors in 219 mice—10.5%), but, in addition, the 219 control pBr mice developed 7 liver tumors (3.2%) and 4 adenocarcinomas of the mammary gland (1.8%). Mice of the JK strain are very resistant to spontaneous tumors. None was observed in 50 females. In a larger series studied over many years, however, lung adenomas and leukemias were occasionally seen (incidence less than 5%).

The experimental mice (the 2NHO) were weaned at thirty or more days of age and placed in boxes usually containing two females with two males. When 60 to 70 days of age, they were injected subcutaneously in the right flank with one milligram

of methylcholanthrene dissolved in 0.1 cc. of sesame oil. The mice were permitted to raise young. Following sixty days the mice were examined weekly for tumors and were killed when tumors at the site of injection or elsewhere on the surface were firm and increasing progressively in size. When mice became emaciated or showed signs of debility they were killed. Autopsies were performed on all mice and all tumors were recorded, fixed in Bouin's solution, sectioned, and stained with hematoxylin and eosin.

The mice were continually subjected to selection toward resistance to all tumors. This was done by keeping the descendants of the most resistant pair of mice in each generation.

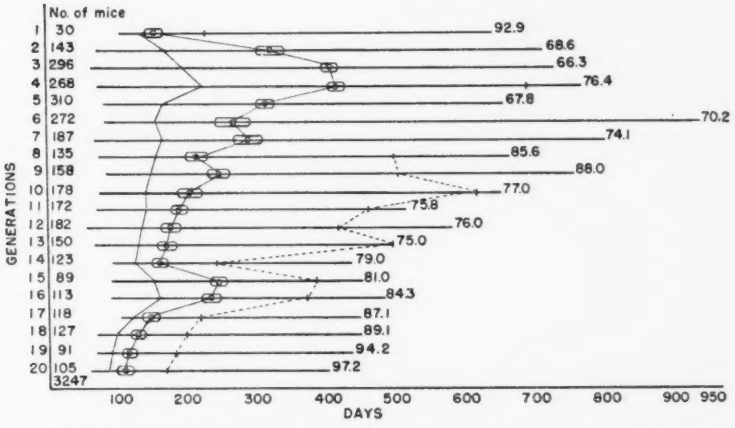

FIG. 2. Data on fibrosarcomas presented by method of quartile distribution for latent periods. Each horizontal line represents a generation. The number of mice, the earliest appearance of a tumor (the left end of the line), the 25%, 50%, and 75% points, and the latest appearance of the tumor (the right end of the line) are given. The probable error of the median is indicated by an oval around the 50% point. Total percentage of tumors is indicated by number at the right end of the lines. Latent period is given on the base line.

Mice of the ancestral pBr mice had been injected with methylcholanthrene between the F_4 and F_9 generations before the outcross to the JK stock had been made. The pBr descent had an average latent period for chemically induced fibrosarcomas of 201 days. The JK ancestry had not been treated with methylcholanthrene, and the mice were in the F_{30} generation when they were outcrossed to mice of the pBr descent.

RESULTS

Four types of lesions were encountered in the mice injected with methylcholanthrene. These were (i) fibrosarcoma at the site of the injection of the carcinogen, (ii) adenoma of the lung, (iii) a gastric lesion just anterior to the pylorus similar to the Brs lesion,[4, 9] and (iv) a papilloma of the forestomach, similar to the one occurring in the pBr subline.[1]

None of these tumors and lesions is unique. They have been encountered in mice of the several selected sublines of NHO descent that were subjected

to methylcholanthrene. The most pronounced difference in 2NHO mice compared to the other descents of the NHO strains is the reduction in the number of the different types of tumors encountered in the original sublines.[8] The outcross to the JK mice may have had something to do with this reduction.

Data on the incidence of fibrosarcoma are given in Figure 2, where the data are presented by the method of quartile distribution for latent periods. Each horizontal line represents a generation. The number of mice, the earliest appearance of the tumor (the left end of the line), the 25%, 50%, and 75% points, and the latest appearance of the tumor (the right end of

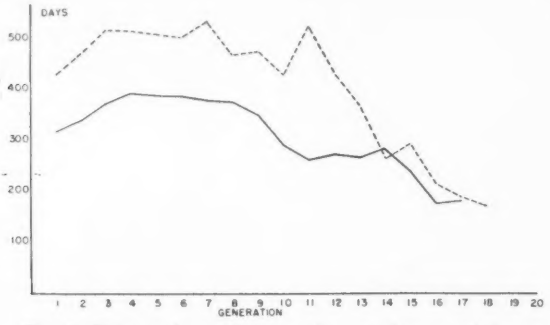

FIG. 3. Data on lung adenomas. Average latent periods are given on vertical line and generations along the base line. Data for females on solid line and males on short dash line.

the line) are given. The probable error of the median is indicated by an oval. This value was determined by the formula,

$$\text{P.E.} = \frac{\text{average quartile deviation from median}}{\sqrt{\text{n}}}$$

where n is the number of mice. The total percentage of tumors is also indicated by the number at the right end of the lines. This chart includes observations on 3,247 mice between the F_1-F_{20} generations.

Data on lung adenomas are given in Table 1 and Figure 3.

Forty-three mice developed a gastric lesion near the pylorus (14 females with an average latent period of 247.2 days; 29 males with an average latent period of 419.6 days). These lesions occurred in mice between the F_3 and F_{14} generations in frequency of 1, 2, 2, 1, 3, 3, 2, 6, 11, 8, 3, 1.

Papillomas of the forestomach occurred in 15 mice between the F_9 and F_{14} generations. Six females had an average latent period of 331.0 days, while 9 males had a latent period of 453.6 days. These lesions were of two types, as follows (i) a pedunculated type, in which the squamous epithelium was hyperplastic and folded upon itself, and (ii) a papillomatous lesion

originating in a broad base. Two of the lesions showed extensive erosion on the surface. In these there was a pronounced foreign body reaction and a downgrowth of epithelial strands. In one case the epithelial cells contained many eosinophilic bodies in their cytoplasm.

Discussion

There was an increase in the average latent period for the occurrence of fibrosarcomas at the site of the injection of methylcholanthrene between the

Table 1

Incidence and Latent Period for Development of Lung Adenomas in 2NHO Mice

Generation	*No. mice*	*Lung adeno-mas*	*% Lung adeno-mas*	♀ *No.*	*Aver. latent period*	♂ *No.*	*Aver. latent period*
F_1	30	8	26.7	3	334.7	5	430.6
F_2	143	35	24.4	14	344.3	21	468.5
F_3	296	76	25.6	33	368.0	43	513.7
F_4	268	59	22.0	23	395.2	36	513.3
F_5	310	57	18.4	21	391.4	36	511.4
F_6	272	50	18.2	24	391.1	26	504.6
F_7	187	41	21.9	19	385.4	22	540.5
F_8	135	34	25.1	18	378.8	16	467.9
F_9	158	43	27.2	16	350.6	27	471.1
F_{10}	178	25	14.0	10	298.8	15	431.6
F_{11}	172	24	13.9	9	257.6	15	529.0
F_1-F_{11}	2149	452	21.0	190	367.0	262	499.8
F_{12}	182	14	7.7	4	271.7	10	437.1
F_{13}	150	8	5.3	5	262.6	3	364.0
F_{14}	123	11	8.8	3	292.3	8	283.0
F_{15}	89	7	7.7	5	248.4	2	297.5
F_{16}	113	3	2.6	1	180.0	2	210.5
F_{17}	118	4	3.4	2	187.5	2	193.0
F_{18}	127	1	0.7	0		1	175.0
F_{19}	91	0	0.0	0		0	
F_{20}	105	0	0.0	0		0	
F_{12}-F_{20}	1098	48	4.4	20	253.7	28	332.3
F_1 -F_{20}	3247	500	15.4	210		290	

F_1 and F_4 generations. This is the period of most rapid decline in heterozygous combinations of genes as well as of a decline of heterosis. In fact the two curves, (i) increase in latent period of tumors and (ii) reduction of heterozygosity, are quite similar and there may be some causal relationship between the two; however, the data on induced fibrosarcomas beyond the F_4 cannot be so readily interpreted. Beginning with F_5, there was a reversal of susceptibility to chemically induced fibrosarcomas, which

resulted in the appearance of these lesions earlier and earlier in mice of successive generations. This phenomenon occurred in spite of a continual selection toward resistance to chemically induced tumors. The trend toward earlier appearing tumors was disturbed in mice of the F_{15} and F_{16} generations for some unknown cause. However, there was a return to a trend of earlier appearing fibrosarcomas in mice of the F_{17} generations, which continued to the F_{20} when the experiment was terminated.

The average latent period for lung adenomas increased in both males and females between the F_1 and F_4 generations (334.7 days to 395.2 days for females and 430.6 days to 513.3 days for males) (Figure 2). It appears, therefore, that the appearance of this tumor was also responding to selection toward resistance to tumors. However, between the F_4 and F_9 generations the latent period for lung adenomas appeared to be constant in mice of the two sexes. Beyond the F_{10}, lung adenomas appeared earlier with a subcutaneous injection of methylcholanthrene than they did in the controls. It is also evident that between the F_1 and F_{11} generations the incidence of lung adenomas was above the spontaneous occurrence of the same neoplasia —21.0% compared to 9.6% (Table 1). However, beyond the F_{11} the incidence of lung adenomas appearing in mice injected with methylcholanthrene was actually below the incidence occurring spontaneously (4.4% compared to 9.6%, Table 1). The sex difference for the appearance of lung adenomas in the early generations F_1-F_{12} was also lost in mice of the advanced F_{13}-F_{18} generations. No lung tumors were found in methylcholanthrene-treated mice during the F_{19}-F_{20} generations. These facts can be explained partially by the appearance of fibrosarcomas which killed the animal before there was time for a lung adenoma to develop, and it is not desirable to conclude, therefore, that methycholanthrene had any inhibiting effect on the appearance of lung adenomas in mice beyond the F_{11} generation.

The two types of gastric lesions, one near the pylorus and the other in the forestomach, have never been found in control 2NHO mice. The significance of their appearance following the subcutaneous injection of methylcholanthrene is not known. In this series, these lesions only appeared during the intermediate generations of inbreeding between the F_3-F_{14}, when the average latent period for the occurrence of fibrosarcomas was sufficient for some of the animals to reach the advanced age at which the gastric lesions could appear (363.5 days for the pyloric lesion and 404.5 days for the forestomach polyp).

Lung adenomas appeared much earlier in mice following the subcutaneous injection of methylcholanthrene. The controls developed lung adenomas with an average latent period of 776.3 days (latent period 747.2 days for females and 836.5 days for males), whereas the methylcholanthrene-treated mice developed lung adenomas on an average of 499.8 days for males between F_1 and F_{11} and 332.3 days for males between F_{12} and F_{20}; 367.0

days for females between F_1 and F_{11} and 253 days for females between F_{12} and F_{20}.

Summary

A total of 3247 mice of the 2NHO descent have been injected subcutaneously with one milligram of methylcholanthrene at sixty days of age. The mice belonged to the F_1-F_{20} generations. A selection toward resistance to all chemically induced tumors was constantly employed. Fibrosarcoma at the site of the injection of the carcinogen and lung adenomas appeared to respond to selection between F_1-F_4. However, beginning with F_5 there was a reversal of susceptibility to these two tumors in spite of selection toward greater resistance. Adenomatous lesions near the pylorus and papillomas of the forestomach appeared in mice only during the intermediate generations F_3-F_{14}. Following a period of delayed latency for the appearance of fibrosarcomas as a result of selection toward resistance to such tumors, there was a progressively earlier appearance of such tumors counter to the trend of selection providing methylcholanthrene had been injected into both parents over many generations. This phenomenon of reversed susceptibility to induced tumors has occurred in *all* selected lines and must be considered the rule.

References

1 Bagshaw, M. A. and Strong, L. C.: The occurrence of tumors of the forestomach in mice after parenteral administration of methylcholanthrene: a histopathologic and genetic analysis. J. Nat. Cancer Inst., 1950, *11*, 141.

2 East, E. M. and Jones, D. F.: *Inbreeding and outbreeding.* Philadelphia, J. B. Lippincott and Co., 1919.

3 Kreyberg, L.: *The genetic and constitutional aspects of spontaneous and induced tumors.* Madison, University of Wisconsin Press, 1938.

4 McPeak, E. and Warren, S.: The pathology of gastric carcinoma in mice. J. Nat. Cancer Inst., 1947, *7*, 309.

5 Strong, L. C.: The nature of susceptibility to cancer in mice. J. Hered., 1934, *25*, 119.

6 Strong, L. C.: A genetic analysis of the induction of tumors by methylcholanthrene. With a note on the origin of the NH strain of mice. Am. J. Cancer, 1940, *39*, 347.

7 Strong, L. C.: Genetic analysis of the induction of tumors by methylcholanthrene. XII. The effects of selection toward resistance. Yale J. Biol., 1946, *18*, 145.

8 Strong, L. C.: Mice, men and malignancy. The George Chase Christian Lecture, University of Minnesota, 1946. Minnesota M., 1946, *29*, 413.

9 Strong, L. C., Collins, V. J., and Durand, E. A.: A genetic analysis of the induction of tumors by methylcholanthrene. IV. The probable remote induction of various types of gastric lesions. Cancer Res., 1943, *3*, 21.

10 Strong, L. C. and Sanghvi, L. D.: Effects of selection on chemically induced tumors in mice (in press).

PUBLIC HEALTH ASPECTS OF ATOMIC DISASTER*

GEORGE JAMES

No conceivable definition of public health can fail to include defense against atomic attack. The very word "public" implies a series of problems facing numerous individuals more or less simultaneously; "health" means the prevention of death or illness and the restoration of the afflicted. The millions in our possible target areas are the "public," and the 100,000 to 500,000 possible casualties following attack by each atomic bomb are the "health" problem. The program for the prevention of death or injury, and the prompt treatment of those afflicted have a top priority on the public health agenda in America today. The solution differs in no significant way from our attack upon familiar public health problems. It is based upon the same time-tested and proven plan, including an accurate determination of our needs, the application of what resources are available or can be made available, and the education of our citizens to understand the problem and act purposefully to meet the challenge.[1]

Preparation for atomic disaster as a public health program

The need for a program for defense against atomic attack is quite apparent, and it is not my purpose to review again the grim logistics of death and injury. Although the exact date of atomic attack upon our land remains with the enemy, if it is known at all, we do possess some knowledge of the apparent effect of this weapon upon our cities. It is as if we knew that an epidemic might strike within the next few years, with its devastation directed essentially against our major cities. Such knowledge, imperfect as it might be, would yet justify an all-out attack on the problem by forces ordinarily channeled against our usual killers, such as tuberculosis, cancer, and heart disease. This warning we have already received with respect to atomic attack. The present world unrest, the knowledge that an unfriendly nation possesses the atomic bomb, the statements of the military—all justify forearming, else our good fortune in being warned will have been wasted. That is why state health departments have now accorded civil defense a top priority, and why efforts have been begun and are now rolling in high gear to provide the needed resources. That is why every effort is being utilized to influence each citizen to understand the problem and accept voluntarily his responsibilities for protective action.

What, then, are the resources with which we prepare to accept the

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challenge of atomic attack? The atomic bomb produces no new and mysterious illness. Medical science has long been aware of the effects of total body radiation, of severe burns, of shock, of extensive wounds, and crushing injuries. Scientific advances have occurred during the past war which have resulted in the most amazing record in the history of military medicine. We are on the threshold of recommending the widespread use of certain cheap, readily stockpiled, and highly effective plasma substitutes for the treatment of shock; we will have available a simple dressing for the local treatment of burns, and new wide-spectrum antibiotics to combat infection. We have a new respect for the value of whole blood which has accompanied the increased willingness of our citizens to act as donors. We enter this emergency with ample knowledge of the required medical techniques. We find the necessary supplies and equipment already invented, and their use, on the whole, well justified by adequate scientific research.

A program for civil defense

Yet, resources, while available in the absolute sense, are of little value to the attacked city which has not received its share. Medical knowledge which is isolated in ivory-towered research institutions will save few lives. In addition, much more is required in the way of influencing community attitudes toward active and adequate preparation for civil defense. Mention will be made of some of the more important attempts which are being made in New York State to meet these problems—to meet them by means of a state-wide civil defense public health program.

Organization for civil defense. Our organization for civil defense[4] is based upon the principle of decentralization. Standing orders will permit field services to begin operations immediately after attack as required by the local situation. Serious delay will result if central control must plot the area of destruction and then order specific field units into action to sites selected only after the receipt of reports from the front. Any interruption in the chain of communications will result in lack of service at the time when it is needed most. True, central control must be prepared to correlate the field efforts, close the gaps, relieve workers at forward posts, and secure and channel aid from beyond the attacked city. Yet, personnel arriving early on the scene, if they have been trained in their functions, can be saving lives long before an exact appraisal of the conditions in the devastated area can be made. This decentralized plan implies large numbers of highly trained medical volunteers, each capable of working together with any other individual who can round off the complement of his squad. For this reason, training, practical experience, and drill of medical volunteers are afforded a high priority in our civil defense program.

The organization for medical defense of a target area is based upon a sector pattern: The city is divided into several wedge-shaped areas radi-

ating out from that point in the city where a bomb explosion might do the most damage. The sector is under the direction of a supply and assignment depot located several miles beyond the periphery of town and it is the station from which mutual aid is channeled toward the aid stations and hospitals. The actual control for first-aid activities remains with the secondary aid stations. These are scattered throughout and beyond the target area, one every two miles in each direction, and serve as the mobilization point for first-aid personnel within the general target area. After attack, teams of physicians, dentists, medical aides, nurses, and messengers will travel from this station toward the devastated area setting up first-aid stations where they are required, as many as are found needed, and with such quantities of personnel as are available. Later, additional personnel will arrive from unattacked areas. As first-hand reports are received from the aid stations, the organization can be solidified, the efforts coördinated, the work rendered more efficient. But during the early hours it is the secondary aid post that will be in contact with its own daughter first-aid posts, acting to relieve their pressure by receiving ambulatory casualties from them, giving litter cases the additional care required prior to hospitalization, and acting as a triage center.

Standardization of medical treatment. If the health officer would attempt to combat a widespread epidemic with limited forces, he would be wise to limit his armamentarium to a few cheap, effective, yet easily learned procedures. So it must be in civil defense. No matter how many persons make up our medical teams for first aid there will be many more injured during the first few hours than can receive immediate care. During the last World War, it was found that delay in providing prompt treatment for the seriously injured could result in irreversible shock. Speed is therefore essential; a physician cannot take hours to debride and apply vaseline gauze dressings to a complicated burn case or to sew up minor lacerations while dozens of seriously wounded are hour by hour approaching that period of irreversible shock. Much of the treatment must be done by nonmedical personnel, and there is a limit to the complexity of treatment procedures which they can learn and learn well. Moreover, it is beyond the capacity of our resources to provide dozens of alternate treatments, each with its particular devotees among the medical profession. For these reasons, under the guidance of the State Medical Society's Committee for Emergency Preparedness, the nine medical schools in New York State nominated their outstanding men to panels dealing with shock and fluid treatment, treatment of fractures, burns, general surgical trauma, radiation illness, and anesthesia, narcotics and related drugs. The recommendations[5] of these panels were ironed out by a conference committee composed of two men from each group. The courage and grasp of the problem shown by these outstanding physicians were indeed gratifying. Orthopedic surgeons agreed that at improvised emergency hospitals fractures might be reduced without the use of x-ray

facilities, that instead of stocking many varieties of special fracture equipment, they could get by with little more than Thomas splints, cotton wadding, and 5-inch plaster bandage. Burns are to be dressed with the simplest of materials such as the new Army burn dressing or even clean sheets over which turkish towels are placed for bulk. There was almost complete agreement that under emergency conditions nonmedical personnel can be trained to do numerous procedures at present exclusively within the domain of the physician.

Training of physicians and medical volunteers. Refresher courses for physicians have already been held in New York State, acquainting them with these panel recommendations. To date, over eighty such courses have been held under the auspices of county medical societies, and over six thousand of our upstate physicians have attended one or more of the sessions.

The training of physicians will not in itself be enough to insure adequate first-aid care for the expected number of the injured. Any available pair of hands fairly well trained in the simple techniques for the control of hemorrhage will save lives which would be lost if our sole resource was a skilled surgeon five miles or even five blocks away. Moreover, so great will be the pressure of first aid upon physicians that as many of the treatments as possible must be delegated to nonmedical personnel. Our plan provides for three types of submedical skill. Those who are dentists, veterinarians, or medical students can perform most of the minor surgery, intravenous work, and special procedures such as bone marrow transfusions. Nurses, too, will assist in these functions, taking over in addition the applications of dressings, splints, and the giving of parenteral medications. The last group is that of medical aides which includes stretcher bearers, ambulance drivers, first-aid workers, and nurses' aides. These will function in any capacity required by the exigencies of the situation.

Training for dentists, veterinarians, medical students, and nurses will be aimed at adapting their skills to the new techniques they will be called upon to perform. The medical aide group is being urged to take the full 22-hour first aid course offered by the American Red Cross. Much thought has been given to the best method of additional teaching of special skills and important organizational patterns. Probably the final result will be the establishment of several training centers throughout each city where all the medical volunteers can meet together periodically to learn the organization and operations, receive more intensive training in their duties, and, by means of actual practice drills, learn the divisions of responsibility. Some of this training should be given in a hospital emergency room so that nonprofessional groups may become accustomed to the sight of injured patients and gain that reassurance which comes from knowing that one's efforts in an unfamiliar field are successful.

Training of the citizen. Because emergency medical personnel cannot promptly reach all persons injured in an atomic explosion, each citizen should know the principles of first-aid care for his injuries and for those of his neighbor. In New York State such an eight-hour program of instruction is being taught in four sessions of two hours each.[3] A select corps of instructors has been trained in each of the six state regions and these, in turn, are organizing and teaching a large number of local instructors. Our goal is to give this course to all citizens of high school age or older, and particular emphasis will be placed on reaching those who reside in target areas.

As is true in all public health programs, prevention is the most effective of all control measures, and the greatest saving of life will occur if each citizen takes the proper measures after the red alert. Hence, programs in public education are deemed of the utmost importance, not only in the prevention of disability and loss of life, but also in reducing panic, improving morale, and allowing for the successful operation of plans for emergency field medical services.

Psychological preparedness. Public indifference to the possibility of disaster from the atomic bomb, or overanxiety concerning its terrors, are alike in predisposing toward panic after attack, while interfering with effective efforts to prepare for medical defense. If we can encourage exactly that amount of anxiety which will lead to purposeful activity for defense, we will also be successful in immunizing our population against widespread panic.[3] Our citizens are informed about the true facts of atomic power and its destructive effect, yet they are also told how to volunteer for service, what our organizational plan will be, how they must be trained to care for themselves and others. We hope to instill a feeling of responsibility and self-reliance by making this training realistic. We expect to inculcate a feeling of identity with a state-wide civil defense organization instead of a small group which may become disorganized after attack. We plan to hold practice drills so as to make their functions after attack more routine, more an integral part of their habitual activity, and consequently less terrifying and panic provoking.

Rôle of medical specialists. With the possible exception of New York City, no target area in our state can be self-sufficient after an attack by even a single atomic bomb. In fact, we have estimated that almost one half of the physicians in upstate New York will be required to give adequate care to the injured resulting from a sneak attack upon one of our major cities. For this reason, little or no distinction can be made between types of physicians during the first 24 to 48 hours after disaster. Each must do his part in field station, emergency, or permanent hospital as determined by the local need at each specific time. We, therefore, plan no *specific* recruiting drives for first-aid physicians, traumatic surgeons, or orthopedists. Each physician in New York State, physically capable of service after attack, is

being recruited for a single entity, *medical defense*. His actual rôle after attack will vary less with his specialty than with the specific problem. We can make no promise to him now as to the limits of his travel, professional utilization, or site of activity.

This means that during the first phase after attack most industrial physicians and surgeons will work, as will other physicians, primarily in first-aid stations. Some, it is true, may begin service in improvised or existing hospitals to be joined later by those who first performed emergency service in field posts. Since first-aid stations will be set up in structures within the disaster area near large concentrations of injured, it will be a relatively rare occurrence that an industry with its medical complement will find itself so located as to be able to utilize its facilities effectively as an aid station. Nevertheless, we are recommending that industries stock a small supply of first-aid equipment, estimated on the basis that 40 per cent of their employees may become injured. After attack, even if the industry is not so situated as to be within easy litter carry of the bulk of the casualties, its supplies can be drawn upon during the early period before the supply service can effectively tap regional depots.

In the days remaining between now and possible attack the industrial physician has several significant functions to perform. In New York State he has been urged to take the leadership in selling to industry the importance of on-the-job training in first aid for all employees. He must do his part in recruiting volunteers to serve as medical aides, nurses, and nursing aides, and accept his share of the responsibility for training them. Because of his knowledge and skill in traumatic surgery and mass medical care, his value to the program of postgraduate medical teaching of physicians should be emphasized. He should enroll in civil defense and be prepared to accept any medical task required. In short, while we can recognize no large group of physicians as exclusive from the standpoint of prior assignment for duty after attack, we must utilize vitally needed abilities possessed by industrial physicians in forwarding our programs of preparedness for atomic warfare.

Conclusion

The specter of atomic attack is a grim one; the devastation of our major cities will be great. Although no one in this country has first-hand experience with this particular menace, we believe that the tested and proved public health principles can be applied to minimize its effect. Determination of our needs has been based upon that Army data which has been interpreted and released. Our resources in men and material have been studied, standardized, and are being made available in practical fashion to our target areas. Attempts are well under way to secure acceptance of new ideas, new programs, and new activities on the part of each citizen. Some anxiety is

being encouraged, but in every case an attempt is made to indicate the correct useful path toward reassurance.

Present evidence points toward our having the menace of atomic attack with us for a long time to come. The solution to the problem of preparation for medical defense against the atomic bomb must therefore remain a top priority health program. Alert to changes in its scope, ever ready to re-evaluate our resources, critical of our progress, mindful of its effect upon the minds and activities of our citizens, New York State expects to meet this challenge with even greater energy and coöperative effort than has yet been applied to the solution of a major public health problem.

References

1 James, George: Practical application of the principles of health department program planning to local tuberculosis control. Am. J. Pub. Health, 1950, *40*, 1219.

2 Janis, I. L.: *Psychological aspects of vulnerability to atomic bomb attacks.* Santa Monica, California, The Rand Corporation, 1949. 63 pp.

3 New York State Civil Defense Commission. *Guide for self-help and neighbor help for the injured.* New York, 1951. 44 pp.

4 New York State Civil Defense Commission. Information Circular No. 10. New York, 1950. 34 pp.

5 New York State Department of Health. Health News. Special Medical Defense Issue. 1951, *28*, 2.

HYPOTHALAMIC CONTROL OF FOOD INTAKE IN RATS AND CATS*

BAL K. ANAND† AND JOHN R. BROBECK

Marked variations in food intake have been described in various species following injury to certain parts of the hypothalamus, including an increased food intake or hyperphagia caused by lesions in the medial hypothalamus, especially lesions in or ventrolateral to the ventromedial nucleus; this hyperphagia leads to obesity.[6–8, 14–16, 20, 24] A decrease or complete inhibition of food intake has also been reported as an incidental finding in animals with hypothalamic lesions, by Hetherington and Ranson[16] in rats, and by Clark, Magoun, and Ranson[10] during their study of temperature regulation in cats. Similar observations were also made in cats by Ingram, Barris, and Ranson,[19] by Ranson[22] in monkeys, and by Anand and Brobeck[3] in certain rats which were being prepared for studies of food intake and activity. The present investigation, therefore, was undertaken as an attempt to localize in the hypothalamus the areas the destruction of which leads to diminution or failure of eating with emaciation, as destruction of certain other areas leads to overeating and obesity. As a result of these studies a small, well-localized area has been found in the lateral hypothalamus; the bilateral destruction of this area is followed by a complete absence of spontaneous eating.[2] This area has been tentatively designated as a "feeding center." An attempt has also been made to discover whether there is any correlation between different areas of the hypothalamus in the regulation of food intake.

Materials and Methods

In a series of 94 female albino rats of the Sprague-Dawley strain, electrolytic lesions were placed in different areas of the hypothalamus with the aid of the Horsley-Clarke instrument as adapted by Clark[9] for use on the rat. Evipal was used for anesthesia (12 mg./100 g. body weight). The lesions were made with a unipolar electrode, by a direct current for 15 seconds, its intensity ranging from 0.8 to 2 milliamperes depending upon the size of the lesion desired. It should be noted here that after the milliammeter had been calibrated properly, the lesions produced with a current of 2 milliamperes were invariably found to be much larger than those reported by Brobeck, *et al.*,[7] and by Hetherington and Ranson[16, 17] with the same current.

For placing small, well-localized lesions, the hypothalamus was divided according to the Horsley-Clarke coördinates into discrete points which were separated from each other by 1 mm. in the rostro-caudal planes, and by ½ mm. in the lateral or parasagittal planes (See Figure 18 and Table 2, below). The area between the level of the para-

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ventricular (filiform) and the supraoptic nuclei, rostrally, and the premammillary and arcuate nuclei, caudally,[21] was divided into five planes, separated from each other by 1 mm. In female albino rats weighing about 250 g., the rostral of these planes was 8 mm. (A 8) and the caudal was 4 mm. (A 4) anterior to the plane of the ear bars.* A 6 corresponded with the central portion of the ventromedial nucleus and A 7 and A 5 with its rostral and caudal limits. The five parasagittal planes (R & L ½, 1, 1½, 2, 2½) were measured from the midline, the fourth (R & L 2) corresponding with the extreme lateral parts of the lateral hypothalamic nuclei, while the fifth (R & L 2½) was slightly outside the lateral limits of the hypothalamus. Horizontally, most of the points chosen were located in a plane which is about 1 mm. above the base of the brain and 1 mm. below the ear bars of the Horsley-Clarke instrument, i.e., H —1. (The base of the brain at this level is in the H —2 plane of our instrument.) A few lesions were also made in the horizontal plane of the ear bars (H 0), and others in the plane 2 mm. below the ear bars (H —2).

The rats were kept in a room where temperature was relatively constant at about 80° F. They were fed ad libitum on a high-fat diet,[13] with the addition of 10 ml. of concentrated cod liver oil, supplemented with 100 mg. of vitamin K and 300 mg. of vitamin E per 100 g. of diet. "Sure B" salt mixture (5%) was used in the diet.

A daily record was kept of food intake and the weight of the rats. Food was always present in the cages, but in order to obtain a full description of spontaneous eating behavior, none of the rats was tube-fed even though it failed to eat to the point of complete starvation.

For controls, rats of the same strain, age, and weight were kept under similar conditions.

Results

I. Large lesions in the region of the ventromedial nucleus

In 18 rats, large, bilateral lesions (2 milliamperes for 15 seconds) were placed in and near the ventromedial nucleus. In 14 of these operations the electrode was localized 1 mm. lateral to the midline, about 1 mm. above the base of the brain (H —1), and in the rostro-caudal plane corresponding to the central portion of the ventromedial nucleus (A 6). Twelve of these 14 ate a very little food for a few days (2-7), and then completely stopped eating (cf. rat 11, Table 1). Their weight gradually fell, and they either died of starvation (although food was always present in the cage), or were killed when in a moribund state in order to save the brain for proper fixation. Sections revealed that all of these 12 rats had large lesions in the hypothalamus, which not only destroyed the medial hypothalamus, but also the whole of the lateral hypothalamus at the rostro-caudal level corresponding to the ventromedial nuclei (Fig. 1, rat 11).

The remaining two rats of these 14 (rats 2 and 5), on the other hand, greatly increased their food intake immediately after the operation, and gradually developed typical hypothalamic obesity (rat 2, Table 1). Sections showed that in rat 2, both the medial and lateral regions of the hypothala-

* The authors realize that these figures have meaning only when applied to the instrument as used in this laboratory, but have chosen to identify the coördinates in order to simplify the description of the operations.

mus were destroyed on the left side, but on the right side the lateral part of the lateral hypothalamus was spared (Fig. 2). In rat 5 the lateral parts of the hypothalamus were spared on both sides(Fig. 3, the medial lesions).

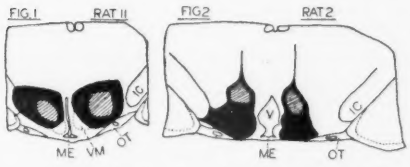


FIG. 1. Section from brainstem of rat 11, showing large lesions of medial and lateral hypothalamus. Rat failed to eat.

FIG. 2. Lesions in hypothalamus of rat 2, with lateral hypothalamic area spared on right side. Rat became obese.

In the other four rats of the series of 18, the placement of the electrode was slightly different. In two of these (rats 40 and 41) the electrode was only ½ mm. lateral to the midline. Rat 40 developed hyperphagia and obesity (Table 1, result of first operation) following lesions which destroyed parts of the ventromedial nuclei and the region dorsomedial to them, while sparing the lateral hypothalamus (Fig. 4, medial lesions.) Rat 41, on the other hand, showed a greatly decreased food intake and a loss of weight for 14 days after operation; its food intake and weight then gradually returned to normal levels, but it did not develop hyperphagia and obesity (Table 1). Its lesions were more dorsal and involved the ventral thalamic and subthalamic regions, extending ventrally only as far as the dorsal limits of the dorsomedial nuclei. The lateral hypothalamus was completely spared.

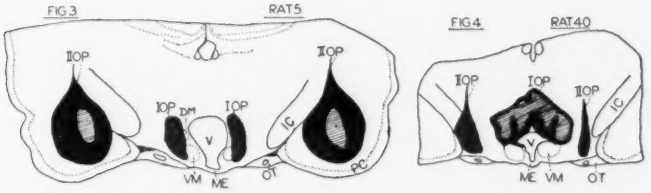


FIG. 3. Medial lesions produced at first operation (I Op.) led to hyperphagia and obesity. At second operation, lesions of amygdala were made for another study.

FIG. 4. Lesions of medial hypothalamus (first operation) induced hyperphagia and obesity. More lateral lesions produced later (second operation) abolished feeding (see section II, A, iii, below).

In one of the two remaining rats (rat 43), the electrode was placed 1 mm. rostral, and in the other (rat 44), 1 mm. caudal to the points of placement in the 14 rats described above. Rat 44 developed hyperphagia and obesity, and the sections showed that although the lesions had completely destroyed the medial hypothalamus including the ventromedial nuclei, the lateral limits of the hypothalamus at that level were not injured. Rat 43 died on the day after the operation with a high fever (colonic temperature of 105° F.) During the one day of its survival it ate normally. Sections

TABLE 1

DAILY FOOD INTAKE IN GRAMS OF RATS WITH HYPOTHALAMIC LESIONS

				Day before or after operation											
Rat	*A**	*R**	*L**	—8	—4	—2	*Op.*	+1	+3	+5	+7	+10	+15	+30	+60
				I. Large, medial lesions											
11	6	1	1	8	7	9.5	6	10	5	0	0	0	Died		
2	6	1	1	8	6	6	17	24	10	27	29.5	23	25	24	12
5	6	1	1	7.5	9.5	9	4	2	4	7	17	18	23	25	
	(First operation)														
	(Second operation, amygdaloid nuclei)						5	7.5	16	27	25	20	18	14	
40	6	½	½	8.5	11	10	22	16	17	10	21	20	22	20	
	(First operation)														
	6	2	2	17	15	13	0	0	0	0	0	0	0	Killed	
	(Second operation)														
41	6	½	½	11.5	12	10.5	2	1	0.5	0.5	0.5	2	7.5	9	9.5
44	5	1	1	10.5	9	12	1	2	3.5	10.5	14	23	29	24.5	14.5
				II A. Small lesions in "feeding center"											
93	6	2	2	11	12	14	0	0	0	0	0	Died			
65	6	2	2	7.5	8.5	9	0.5	0	0	0	0	Died			
86	6	2		7	7.5	8	4	6	9	8	7				
	(First operation)														
	6		2				0	0	0	0	0	Died			
	(Second operation)														
42	6	1	1	10.5	8.5	8	10.5	13	14	14	22	23	26	22.5	
	(First operation)														
	6	2	2	23	18.5	22	0	0	0	0	0	0	0	Killed	
	(Second operation)														
63	6	1	1	10.5	7.5	10	2	4.5	24	31	25	32	22.5	25	
	(First operation)														
	6	2		32	19	20	2	11	26	19.5	31	30			
	(Second operation)														
	6		2		30	24	0	0	0	0	0	0	Killed		
	(Third operation)														
123	6	2	2	9.5	10	13	0	0	2	2	4	6	10.5	8.5	
	(Asymmetrical)														

				Day before or after operation											
Rat	*A**	*R**	*L**	*−8*	*−4*	*−2*	*Op.*	*+1*	*+3*	*+5*	*+7*	*+10*	*+15*	*+30*	*+60*
							II B. Small lesions in adjacent areas								
118	Control			13	11	12		10	8.5	9.5	7.5	8	6.5	8.5	8
98	6	2½	2½	11	7.5	12	*0*	2	6	8.5	11	9	8.5	7.5	8.5
103	6	1½	1½	12	11	10	*7*	2	9	9	12	11.5	13.5	14	13.5
133	6	1½	1½	14	9	13	*0*	1	0	0	2	10	11.5	14.5	13
94	7	2	2	11	8	11	*0*	0	6	7	8	10	7.5	9	9
92	5	2	2	10	14	13	*3*	5	7	5	4	9	7.5	9.5	8
116	6	2	2	8	7	9	*0*	0	13	7	9	8	9	9.5	7.5
	(One mm. dorsal)														
117	6	2	2	11	8	10	*0*	0	3	1	0	0	Died		
	(One mm. ventral)														
							II C. Small lesions in other areas								
102	5	1½	1½	8.5	9	10	*1*	10	8	7	14	13	14.5	18	17.5
109	7	1	1	10	9	11	*14*	20	10	16	18	17.5	18	20.5	19.5
112	5	½	½	11	10	11	*10*	17	10	13	12	14	13	11.5	10.5
100	8	2½	2½	11.5	12	12	*3*	4	7	5.5	8.5	7.5	6.5	7.5	7
111	4	½	½	12.5	13	12	*3*	9	7	7.5	8	9	7	8.5	8
101	4	1½	1½	11	12	12	*0*	3	8	9	9	8.5	5.5	9.5	10
106	4	1	1	11.5	10	12	*3*	10	6	9	9	11	10	11	9.5

* A is distance anterior, R and L distance lateral, in millimeters.

showed a complete destruction of the anterior hypothalamic regions, but the lateral limits of the hypothalamus at the level of the ventromedial nuclei were spared.

Study of sections of this group suggests that if the regions around the ventromedial nuclei are destroyed, while the lateral parts of the lateral hypothalamus at the rostro-caudal level corresponding to the ventromedial nuclei are intact either on both sides (rats 5, 40, and 44), or even on one side (rat 2), the animal develops hyperphagia and obesity. If the lateral parts of the lateral hypothalamus are destroyed along with the medial, then the rat stops eating and dies of starvation. Rat 41, an animal that recovered normal eating, had the lateral hypothalamus of both sides, as well as regions around the ventromedial nuclei, spared completely. Rat 43 received no injury to the lateral parts of the hypothalamus, and ate normally during the day it survived; but since the anterior hypothalamus was destroyed, the rat died of hyperthermia.[10]

In addition to the changes in food intake, certain other observations may be noted here.

(i) All of the rats with large lesions showed marked hyperactivity beginning immediately after recovery from the anesthesia and persisting for about one day.[18]

(ii) Ten rats of this group showed from a slight to a marked somnolence, and in all of these the lesions were found to extend back to the mammillary region.[22]

(iii) Twelve rats of this series showed from a fair to a marked degree of irritability, and five of them were really vicious. The irritability and viciousness decreased on repeated, gentle handling, but did not disappear completely. Rat 5 was the most vicious of the group and would bite at every attempt to handle it. Its viciousness showed no decrease until a second operation was done, when large, bilateral, electrolytic lesions were placed in the lateral group of amygdaloid nuclei (Fig. 3, lateral lesions). Following this operation, surprisingly, the rat was completely tame; after that it did not bite at all.

(iv) Some of the rats that had complete inhibition of eating showed pink porphyrin incrustations around the nose and eyes.[11]

(v) A record of colonic temperatures was kept in 8 of the rats of this group. Except for rat 43, which developed hyperthermia, none showed any change in body temperature.

II. Small lesions

In the remaining 76 rats, only small, well-localized lesions were produced.

A. Small lesions in the lateral hypothalamic area at the level of the ventromedial nucleus.

(i) In five female rats, small, bilateral lesions were produced in the extreme lateral portion of the lateral hypothalamus, using a direct current of 1 ma. for 15 sec. The

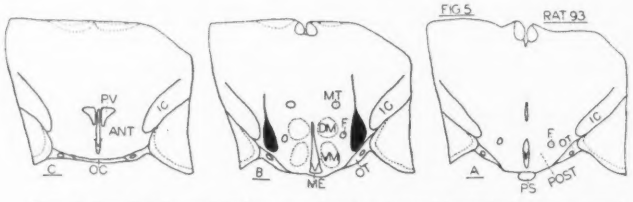


FIG. 5. Small, bilateral lesions restricted to lateral hypothalamus at level of ventromedial nuclei. Feeding was abolished, presumably because of bilateral destruction of "feeding center."

electrode was placed 2 mm. lateral to the midline, about 1 mm. dorsal to the base of the brain (H -1), and at the rostro-caudal level of the central portion of the ventromedial nucleus (A 6) (Fig. 5, rat 93). As the drawings indicate, these lesions were small and well localized, and did not extend either rostrally or caudally beyond the level of the ventromedial nucleus. The operation was immediately followed by a complete cessation of eating, the body weight gradually fell, and after losing between 90 and 130 grams in weight the rats died on the eighth to twelfth postoperative day (Table 1, rats 93 and 65).

(ii) Unilateral lesions were produced in this area of the lateral hypothalamus in three rats with no effect upon eating behavior (Table 1, rat 86). When another unilateral lesion was made in the corresponding area of the opposite side, there followed immediately a complete inhibition of eating. A section from the brain of one of these rats is shown in Figure 6.

(iii) Small, bilateral lesions (Figs. 4 and 7, lateral lesions) were made in the lateral hypothalamic area in nine rats which had previously been made hyperphagic

and obese by more medial, bilateral lesions. The earlier hyperphagia at once yielded to non-eating; these animals, again, did not eat at all, and even though food was always available, they died of starvation (Table 1, rats 40 and 42).

(iv) In four rats, previously made hyperphagic and obese by bilateral, medial lesions, small, unilateral lesions were placed in this lateral area. After a day or two of diminished eating, the animals recovered and behaved as they would have if no such unilateral lesions had been present. In other words, if the rats were still in the "dynamic" stage of obesity, they continued hyperphagic and gaining weight; if they had already reached the "static" phase, they continued at that weight, eating their accustomed amount of food (Table 1, rat 63). Later, when a corresponding lesion was made also on the opposite side (Fig. 8), eating was at once abolished.

(v) In another investigation, carried out to study the effects of lesions in the amygdaloid nuclei, two rats were observed to stop eating after an attempt had been made to place large lesions in the medial group of amygdaloid nuclei. Study of sections revealed that the lesions had encroached upon the lateral hypothalamic area on both

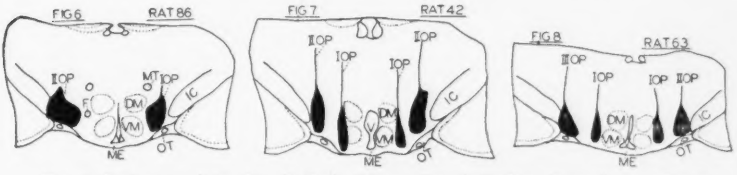


FIG. 6. Lesion on right side (I Op.) did not disturb feeding. Second operation to produce left lesion (II Op.) led to complete absence of feeding.

FIG. 7. Medial lesions (I Op.) induced hyperphagia and obesity. Feeding disappeared following the production of lateral lesions (II Op.).

FIG. 8. Hyperphagia and obesity followed production of medial lesions (I Op.). First lateral lesion (II Op.) did not further alter food intake, but after second lateral lesion (III Op.) the rat ate nothing.

sides (Fig. 7 in Anand and Brobeck[3]). In other animals of the series the eating remained normal; here the lesions were found to be outside the limits of the hypothalamus.

It is thus clear from these operations that bilateral destruction of the lateral portion of the lateral hypothalamus, in the same rostro-caudal and horizontal planes as the ventromedial nucleus, abolishes the food intake of rats, even in animals which have previously been made hyperphagic and obese. None of the animals in which this area of the lateral hypothalamus has been bilaterally destroyed has ever eaten any food during the entire period of its postoperative survival—and this in spite of our many attempts to put food near to or even inside the mouth. In some instances attempts to put the food cup near the mouth were resisted by the rat's pushing the cup away. Changing the diet from the high-fat mixture to "chow" pellets also failed to induce eating.

There was only one rat that recovered normal eating after an attempt had been made to produce lesions of this type in the lateral hypothalamus (Table 1, rat 123). Sections of its brainstem showed that the lesions were asymmetrical, the right one being in the lateral hypothalamus, while the

left one was outside this area (Fig. 9). Thus, in our experience, bilateral lesions in this lateral area can be said to have produced 100% of positive results in terms of failure to eat. The region is well localized and so specific with reference to the Horsley-Clarke coördinates that even small, bilateral lesions symmetrically produced using the appropriate coördinates can be expected always to lead to the desired effect of non-eating.

With the exception of the last days before death when they were emaciated and lethargic, the animals that failed to eat in their early postoperative days looked quite normal and showed a normal amount of activity and of movement about the cage. During this period they appeared to be more irritable than normal, but none of them showed any postoperative hyperactivity nor the somnolence seen after the larger lesions. During their last days some had pink porphyrin incrustations around their mouth and

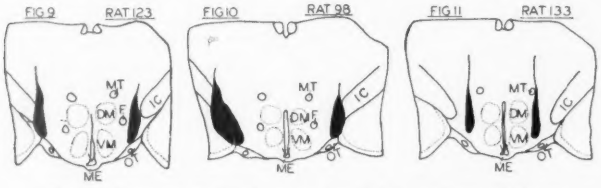


FIG. 9. Asymmetrical lesions sparing left lateral hypothalamus. After depression of food intake lasting 10 days, rat ate normally.

FIG. 10. Section showing lesions situated lateral to lateral hypothalamus; "feeding center" was spared.

FIG. 11. Small lesions in region between ventromedial nuclei and lateral hypothalamic areas. Rat slowly became obese.

eyes. Colonic temperatures, measured in five of them, showed no change from normal.

B. Small lesions in adjacent areas. Small, symmetrical, bilateral lesions were placed in areas adjacent to and all around the area described above and designated as a "feeding center."[8]

(i) In three rats, bilateral lesions were placed ½ mm. lateral to the above mentioned point, i.e., 2½ mm. from the midline. Two of these rats stopped eating for one day, and one did not eat for five days, after which they began to eat and gradually increased their intake to preoperative levels in 5-7 days (Table 1, rat 98). Food intake and body weight then remained comparable to those of normal controls. Sections revealed that the lateral hypothalamus was completely spared on both sides in all three rats, and that the lesions involved variable amounts of injury to fibers of the internal capsule and optic tract, as well as to the tips of the pyriform lobes (Fig. 10).

(ii) In four rats, bilateral lesions were placed ½ mm. medial to the "feeding center," i.e., 1½ mm. from the midline. Two of these rats ate something on each day, although their intake was decreased for 3-5 days, after which it returned to preoperative levels (Table 1, rat 103). In another, feeding ceased for one day and then gradually recovered, while in the fourth rat that ate practically no food for 7 days, feeding then gradually returned to preoperative levels in another 4 days (Table 1, rat 133). When their

postoperative food intake is carefully compared with that of controls (Table 1, rat 118), one can see that the rats with these lesions eventually ate somewhat more than normal, since in unoperated female rats of the Sprague-Dawley strain the daily food intake diminishes when the animal attains a weight of about 250 grams and "plateaus" at this level. In this group of animals with lesions the food intake neither decreased,

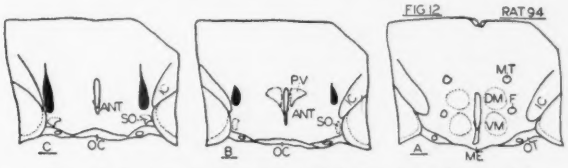


FIG. 12. Lesions in lateral hypothalamus located rostral to "feeding center." After transient depression, food intake became normal.

nor did the weight become stationary but went on gradually increasing for some 3 months as the rats slowly became obese. Sections from three of these rats revealed lesions in the lateral hypothalamus which did not reach out to its extreme lateral parts (Fig. 11). In one rat (rat 103) the lesion on the left side had, in fact, encroached upon the extreme lateral part of the hypothalamus; but on the right side this area was spared.

(iii) In three rats, bilateral lesions were placed in a plane 1 mm. rostral to the "feeding center." All of these animals stopped eating for one or two days, and then gradually recovered (Table 1, rat 94). Postoperative food intake and weight were otherwise comparable to those of controls. Sections revealed lesions in the extreme lateral limits of the hypothalamus in its anterior portion, extending anteriorly to the level of the paraventricular nuclei; but caudally the lesions did not extend back to the level of the ventromedial nucleus (Fig. 12).

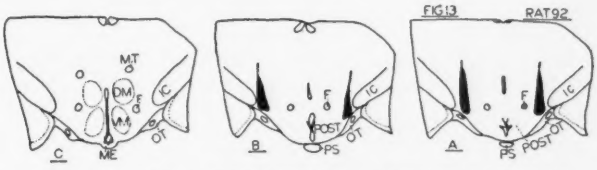


FIG. 13. Lesions in lateral hypothalamus situated caudal to level of "feeding center." Following a temporary depression, food intake became normal.

(iv) Similar bilateral lesions were placed in three rats in a plane 1 mm. caudal to the "feeding center." Two of these rats stopped eating for one day and then gradually recovered, while one had only a decreased food intake for 3-4 days, followed by normal eating (Table 1, rat 92). Postoperative food intake and weight were then comparable to those of the controls. Sections revealed localized lesions in the extreme lateral parts of the hypothalamus in its caudal portion; the lesions did not extend rostrally as far as the level of the ventromedial nucleus (Fig. 13).

(v) In two rats, bilateral lesions were placed in a plane ½ to 1 mm. dorsal to the "feeding center." These rats also stopped eating for two days and then gradually recovered (Table 1, rat 116). Sections revealed lesions which were dorsal to the

lateral area of the lateral hypothalamus; ventrally the lesions did not reach beyond the ventral limits of the dorsomedial nuclei (Fig. 14).

(vi) In two rats, on the other hand, bilateral lesions were placed in a plane ½ to 1 mm. ventral to the "feeding center." In one rat there was an immediate and complete cessation of eating. The other rat stopped eating for two days after operation; for the next three days it ate small and gradually diminishing amounts of food, and then once more completely failed to eat (Table 1, rat 117). Sections revealed lesions in the lateral part of the lateral hypothalamus, which ventrally reached the base of the brain, and dorsally extended up to the level of the dorsomedial nuclei (Fig. 15).

It thus appears that bilateral destruction of areas adjacent to the region described above (A), and as near as ½ to 1 mm. in all directions around it except ventrally, does not produce the same cessation of eating. As a result of these nearby lesions the feeding may either stop or diminish for a few days and later return to normal levels. Or, if the destruction involves

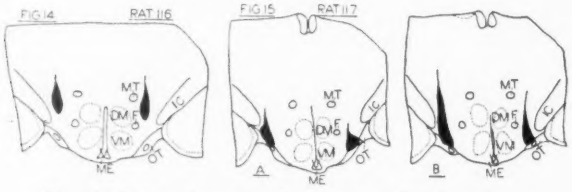


FIG. 14. Lesions dorsal to "feeding centers" of lateral hypothalamus. Rat ate less for a few days, then ate normally.

FIG. 15. Position of lesions placed 1 mm. ventral to others of this series at level of ventromedial nucleus. Injury extended dorsally along needle tracts. Feeding was almost abolished.

the medial areas adjacent to the "feeding center," the animal eats more than normal and gradually becomes obese (see below). Destruction of regions ventral to the "feeding center," on the other hand, also produces cessation of eating, but we have not been able to make lesions that were confined to the base of the brain using this particular technique, since the injury always spreads dorsally along the needle tracks (Fig. 15). Thus, the ventral limits of the "feeding center" cannot be clearly established from our data. The fact that rat 117, with lesions near the base of the brain, did eat some food for each of three days may suggest that the dorsal extension of the lesions rather than their more ventral components was responsible for the failure to eat.

None of the animals belonging to this group, i.e., those with small lesions in areas adjacent to the "feeding center," showed any postoperative hyperactivity, somnolence, irritability, or viciousness. Their colonic temperatures were normal.

C. Small lesions in other areas of the hypothalamus. As described in METHODS, the area of the hypothalamus in the horizontal plane 1 mm. above the base of the brain, bounded (a) medially by the midline, (b) laterally

by a parasagittal plane 2½ mm. from the midline (this plane passes somewhat lateral to the limits of the hypothalamus), (c) rostrally by a plane passing through the region of the paraventricular and supraoptic nuclei, and (d) caudally by a plane passing through the premammillary and arcuate nuclei, was divided into 25 points on each side for placing small, localized, bilaterally symmetrical lesions. The effects of some of these lesions have already been reported under (A) and (B); the operations not already described above yielded the following results.

(*i*) *Lesions producing overeating and obesity.* Bilateral lesions in the areas situated in the tuberal portion of the hypothalamus, between ½ to 1½ mm. from the midline, but not destroying both of the areas in the lateral hypothalamus which we have called the "feeding center," lead to increased eating and a gradual development of obesity, provided that the lesions encroach upon this area at the level of, that is, within the rostro-caudal limits of the ventromedial nucleus (Table 1, rats 102, 109, and 112). Thus, small, bilateral lesions placed ½ mm., 1 mm., and 1½ mm. lateral to the midline,

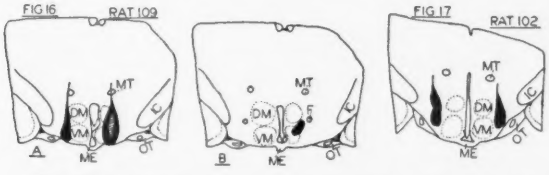


FIG. 16. Lesions in region of ventromedial nuclei, producing moderate hyperphagia and obesity.

FIG. 17. Lesions which induced hyperphagia and obesity although ventromedial nuclei were not directly injured.

and within the rostro-caudal limits corresponding to the A 5, A 6, or A 7 coördinates of the Horsley-Clarke instrument, led to obesity. Although the food intake was definitely more than that of unoperated controls, the increase was not as marked as the hyperphagia produced by the larger lesions described in an earlier paper.[7] In rats with the smaller lesions the development of obesity was also slower, and the body weight did not reach the higher limits attained by rats with larger lesions. For example, in the group of rats with small lesions (see above), where care was taken to see that all of the animals were of the same strain and age, as well as of comparable weight and food intake before operation, certain of the rats after operation showed greater hyperphagia and earlier obesity than others, with no good correlation between these phenomena and the coördinates at the time of operation. Yet when the sections were studied, the differences in food intake could then be correlated with the size of the lesion in this particular area; the larger the lesion, the more marked the increase in food intake. Rat 109 showed the best hyperphagia, was the earliest to develop obesity, and exhibited the largest lesions (Fig. 16). Next in order in this group was rat 102 (Fig. 17).

(*ii*) *Lesions followed by normal eating.* Bilateral lesions outside the limits of the area associated with hyperphagia, and which also did not affect the "feeding centers" in the lateral hypothalamus, did not produce any change in eating (Table 1, rats 111 and 100). For example, none of the rats with bilateral lesions in the more rostral plane of the paraventricular and supraoptic nuclei (A 8) or in the more caudal plane of premammillary and arcuate nuclei (A 4) showed any change in food intake or

increase of weight beyond the normal limits, when the lesions were confined to that particular level. Two animals (rats 106 and 101) with lesions in the more caudal plane (A 4), which did show some increase of food intake, were found at autopsy to have lesions extending rostrally up to the caudal limits of the ventromedial nuclei. Again, bilateral lesions in the lateral planes 2 mm. and 2½ mm. from the midline did not cause any change in food intake except for the bilateral lesions in the "feeding center."

None of the animals of this (C) group, whether they developed obesity or not, showed any postoperative hyperactivity, somnolence (not even those with the caudal lesions), irritability, or viciousness. Regarding their body temperature, all rats had normal colonic temperatures except the animal (rat 83) that died on the day after the operation with a fever of 104.5° F. Sections showed bilateral destruction of a large part of the anterior hypothalamus from the paraventricular nuclei up to the rostral limits of the supraoptic nuclei. This result is analogous to the course of rat 43 (see above) which also died of hyperthermia, with much larger, bilateral lesions in the same area. In the remainder of the animals, showing only small lesions of these rostral areas and injury to only a part of the anterior hypothalamus, there was no change in

TABLE 2

AVERAGE RATE OF DAILY CHANGE IN BODY WEIGHT DURING THE FIRST THIRTY DAYS AFTER OPERATION IN 25 RATS WITH LESIONS AND 3 CONTROLS

Controls	*R & L ½*	*R & L 1*	*R & L 1½*	*R & L 2*	*R & L 2½*	
	♯ *115* +1.3 g.	♯ *110* +1.0 g.	♯ *105* +1.0 g.	♯ *95* +1.0 g.	♯ *100* +1.2 g.	*A 8*
♯ *120* +1.0 g.	♯ *114* +2.1 g.	♯ *109* +5.0 g.	♯ *104* +2.4 g.	♯ *94* +1.3 g.	♯ *99* +1.0 g.	*A 7*
♯ *119* +1.3 g.	♯ *113* +2.0 g.	♯ *108* +3.0 g.	♯ *103* +2.5 g.	♯ *93** —9.0 g.	♯ *98* +1.3 g.	*A 6*
♯ *118* +1.2 g.	♯ *112* +3.0 g.	♯ *107* +2.5 g.	♯ *102* +3.6 g.	♯ *92* +0.9 g.	♯ *97* +1.1 g.	*A 5*
	♯ *111* +1.3 g.	♯ *106* +2.0 g.	♯ *101* +2.3 g.	♯ *91* +1.3 g.	♯ *96* +1.3 g.	*A 4*

R & L denote the lateral coördinates, in millimeters from the midline.
A denotes the distance anterior to the plane of the ear bars, in millimeters.
* Survived only 8 days.

temperature. In another rat (rat 80), a large, unilateral lesion was placed on the right side in the anterior hypothalamus; there was no change in colonic temperature. After 20 days another large, unilateral lesion was placed in the same area of the opposite side, but this did not lead to hyperthermia and the rat survived. Sections revealed large lesions in the same regions as those of rat 83.

Table 2 shows the average rate of daily increase or decrease in body weight during the first thirty postoperative days in a group of 25 animals of the same weight and age, operated upon on the same day, with small, bilateral lesions in each of the 25 areas mentioned above. The table also includes data from three normal rats of the same group which served as controls.

In Figures 18 and 19 an attempt has been made to project the results of bilateral lesions in these various regions onto a diagrammatic representation of one side of the hypothalamus (taken from Krieg[21]).

III. Hypothalamic lesions in cats

During the course of another study, bilateral, electrolytic lesions were produced by means of the Horsley-Clarke instrument in certain areas of the hypothalamus in cats. The following pertinent observations were made concerning food intake.

(i) In two cats, large, electrolytic lesions were produced bilaterally (4 ma. for 30 sec.) in and around the central and medial nuclei of the amygdala[12]; a complete cessation of eating was observed immediately after the operation in one of these cats. She was kept alive for 6 weeks by tube feeding and during this period showed no abnormal somnolence, was quite active, and did not develop rage. Neurological examination revealed no abnormality; vision and smell likewise appeared to be normal. Not only was there no spontaneous eating, but even attempts to put food into the mouth did not elicit eating or swallowing—on the contrary, the food was always ejected from the mouth. The animal was autopsied after 6 weeks, and sections revealed large, bilateral lesions which, in addition to destroying the amygdala, spread medially into the lateral regions of the hypothalamus on both sides. The second cat did not eat spontaneously for 7 days, during which time it was tube-fed. Then she began to eat a little food spontaneously, but the food intake was much less than before operation. Her behavior was in other respects quite normal. Sections showed much less destruction of the lateral hypothalamus than in the first cat.

(ii) In two other cats, bilateral lesions were first made in and around the ventromedial nuclei, resulting in increased food intake and obesity. One of these cats developed a marked degree of hyperphagia, with an average daily food intake (canned dog food) which rose from a preoperative level of 80-90 g. to 400-500 g. She became markedly obese, her weight increasing from 2.2 kg. to 5.8 kg. She was the only cat which developed obvious rage reactions.[20, 5] Eight weeks after the first operation, bilateral lesions were placed in the lateral portions of the lateral hypothalamus in the rostrocaudal plane of the ventromedial nucleus. The animal remained alive for 8 days after operation, and during this period there was a complete failure of eating as well as of drinking. In addition, she showed some degree of somnolence. She was not tube-fed, and died on the eighth day, after her weight had fallen from 5.8 to 4.3 kg. She exhibited no rage during this period. The other cat showed much less increase in its daily food intake following the first operation, its weight increasing from 2.2 to 3.4 kg. in 24 days. She remained friendly and manifested no rage. Lateral lesions were then made, but she lived only for one day after this second operation and suddenly died. During this one day she did not eat or drink.

Discussion

The present study appears to show that both increased food intake (hyperphagia) and decreased food intake or even complete cessation of eating can be produced as a result of certain bilateral hypothalamic lesions. The region the bilateral destruction of which produces hyperphagia and obesity, as localized by the studies of Hetherington and Ranson[17] and Hetherington,[15] includes some parts of the ventromedial nuclei or the adja-

cent areas of the lateral hypothalamus. Lesions more rostral or dorsal which completely spare these nuclei do not lead to hyperphagia and obesity. Those authors have also reported that these nuclei need not be completely destroyed to produce obesity. The results of our study with well-localized, small lesions generally confirm their observations, although we have found that bilateral lesions placed lateral to those nuclei, and completely sparing the nuclei themselves, also bring about obesity. Hetherington and Ranson[37] have observed obesity following bilateral lesions placed caudally—dorsolateral to the premammillary and mammillary regions—which they attributed to the destruction of efferent fibers running from the ventromedial nucleus, or its neighborhood, into the brainstem. Ruch, Patton, and Brobeck[35] likewise obtained obesity in monkeys from still more caudally placed lesions of the ventral part of the thalamus and the rostral portion of the mesencephalic tegmentum. We have not been able to obtain similar results in rats from small caudal lesions in the particular horizontal plane which we have chosen. Of six animals in which caudal lesions were made, only two (rats 106 and 101) showed slight increases of food intake, and sections from the brain of these two revealed lesions extending rostrally up to the caudal limits of the ventromedial nuclei (see group C, (ii), above).

FIG. 18

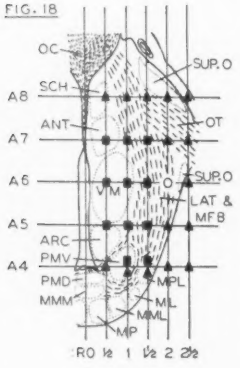
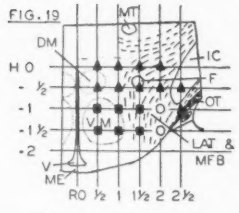


FIG. 19



▲ NORMAL FOOD INTAKE
O NO FEEDING BEHAVIOR
■ HYPERPHAGIA

FIG. 18. Diagram representing right side of rat's hypothalamus (from Krieg[37]), in horizontal plane of ventromedial nucleus. Horsley-Clarke coördinates are superimposed, and feeding behavior of rats with small, bilaterally symmetrical lesions in each area is indicated.

FIG. 19. Diagram showing cross section of right side of rat's hypothalamus at level of ventromedial nucleus, with Horsley-Clarke coördinates superimposed. Feeding behavior of rats with small, bilateral lesions in each area is indicated.

Another, more highly circumscribed, area has been found in the extreme lateral portion of the lateral hypothalamus at the same rostrocaudal plane as the central part of the ventromedial nucleus, bilateral destruction of which leads to complete inhibition of food intake. Unilateral destruction of this area does not produce this effect, and this is in conformity with the observations that unilateral injury to the medial hypothalamus does not lead to obesity, and that stimulation of either side of the hypothalamus usually affects both sides of the visceral nervous system, and probably of the somatic, as well.

As noted above, failure of spontaneous eating for variable periods following hypothalamic injury has already been reported incidentally by

Hetherington and Ranson[18] in rats, by Clark, Magoun, and Ranson[20] and by Ingram, Barris, and Ranson[19] in cats, and by Ranson[22] in monkeys. Although they did not localize the regions responsible for eating, their results in general corroborate ours.

This particular lateral area of the hypothalamus not only contains some portion of the large stellate cells constituting the lateral hypothalamic nucleus, but in addition has in it a large group of fibers from the medial forebrain bundle, from the stria terminalis, and direct amygdalo-hypothalamic fibers, as well as fibers from the ansa lenticularis.[21] Since these same fibers are likely to be destroyed in lesions of the adjacent medial, lateral, rostral, caudal, and dorsal areas, which in our experience did not disturb eating, it is difficult to believe that these particular fibers make up the "feeding center." It may well be that this "center" is composed of neurons lying among these fibers, as the cells of the respiratory center are thought to lie in the reticular formation of the medulla. Adjoining the area of the "feeding center" are other important structures, such as the internal capsule, optic tract, tip of the pyriform lobe, and, a little more laterally, the medial group of amygdaloid nuclei; still further dorsal and lateral are the basal ganglia. Since lesions which have been confined to the lateral hypothalamus without direct injury to these other structures (Fig. 5) have completely inhibited eating, while lesions which destroyed the adjoining structures at the same time sparing at least one lateral hypothalamic area have not abolished eating (Fig. 10; cf. also Anand and Brobeck[3]), it can be inferred that this effect is probably due solely to lesions in the lateral hypothalamus. The behavior of these rats and cats suggests that the lesions have in some manner removed what Adolph has called the "urge to eat."[1]

It further appears from these observations that the more medial hypothalamic structures (where lesions cause hyperphagia) exert their influence on food intake through the "feeding center," possibly by means of inhibitory fibers running laterally into that center. Thus, an animal fails to eat following destruction of the "feeding centers" of both sides, while if the "feeding centers" (or even one of them) are intact and the more medial mechanism is removed by destruction of the ventromedial nuclei or their efferent fibers, the animal exhibits a state of hyperphagia. This concept is also in accord with the observation that the degree of hyperphagia and the rapidity with which obesity develops depend upon the size of the lesions in the more medial region, in other words, upon the number of inhibitory fibers damaged. On the other hand, combined bilateral lesions of the medial and lateral mechanisms, together, produce the same effect as lateral lesions alone, that is, failure to eat.*

Summary

A small area has been localized in rats in the extreme lateral part of the lateral hypothalamus, in the rostro-caudal plane corresponding to the central

part of the ventromedial nucleus. Bilateral destruction of this area leads to a complete cessation of eating. A unilateral lesion has no such effect. Bilateral lesions involving either the ventromedial nuclei, or the region between them and the lateral area (but not involving the lateral area), produce hyperphagia and obesity. Bilateral lesions in other areas of the hypothalamus do not produce a change in food intake, with the exception that injury to regions adjacent to the lateral area may induce diminished eating for a few days after operation. If the animal is made obese by medial lesions, eating ceases when the lateral areas are destroyed bilaterally. This lateral area has been called a "feeding center"; it may be responsible for the central hunger reaction or the urge to eat, while the ventromedial nucleus or some structure in its neighborhood may be capable of exerting an inhibitory control over the "feeding center" through fibers which run laterally into the lateral hypothalamic area.

Similar observations have been made in cats.

Abbreviations for all Figures

ANT,	nucleus hypothalamicus anterior
ARC,	nucleus arcuatus
DM,	nucleus hypothalamicus dorsomedialis
F,	fornix
IC,	internal capsule
LAT,	nucleus hypothalamicus lateralis
ME,	median eminence
MFB,	medial forebrain bundle
ML,	nucleus mammillaris lateralis
MML,	nucleus mammillaris medialis pars lateralis
MMM,	nucleus mammillaris medialis pars medialis
MP,	nucleus mammillaris posterior
MPL,	nucleus mammillaris prelateralis
MT,	mammillo-thalamic tract
OC,	optic chiasma
OT,	optic tract
PC,	pyriform cortex
PMD,	nucleus premammillaris dorsalis
PMV,	nucleus premammillaris ventralis
POST,	nucleus hypothalamicus posterior
PS,	pituitary stalk
PV,	nucleus paraventricularis (filiformis)
SCH.,	nucleus suprachiasmaticus
SO & SUP. O,	nucleus supra-opticus
V,	third ventricle
VM,	nucleus hypothalamicus ventromedialis
I, II, & III OP.,	first, second, and third operation respectively
A4 to A8,	4 to 8 mm. anterior to the ear bars of Horsley-Clarke instrument
L ½ to L 2½,	½ to 2½ mm. lateral to the midline
H-½ to H-2,	½ to 2 mm. ventral to the ear bars of the Horsley-Clarke instrument

* Confirmative evidence in support of the existence of a "feeding center" in this region has been obtained by Anand and Delgado (1951) in cats. The appropriate area of the lateral hypothalamus has been stimulated chronically in unanesthetized animals by means of implanted electrodes. Food intake increased greatly during the days when the stimulation was carried on, and then fell once more to normal levels when the stimulation was discontinued.

References

1 Adolph, E. F.: Urges to eat and drink in rats. Am. J. Physiol., 1947, *151*, 110.

2 Anand, B. K. and Brobeck, J. R.: Localization of a "feeding center" in the hypothalamus of the rat. Proc. Soc. Exp. Biol., N. Y., 1951, *77*, 323.

3 Anand, B. K. and Brobeck, J. R.: The food intake and spontaneous activity of rats with lesions in the amygdaloid nuclei. J. Neurophysiol., to be published.

4 Anand, B. K. and Delgado, J. M. R.: Chronic stimulation of hypothalamus in unanaesthetized cats with special reference to their food intake. 1951 (in preparation).

5 Bard, Philip and Mountcastle, V. B.: Some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behavior. Res. Pub. Ass. nerv. ment. Dis., 1948, *27*, 362.

6 Brobeck, J. R.: Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev., 1946, *26*, 541.

7 Brobeck, J. R., Tepperman, J., and Long, C. N. H.: Experimental hypothalamic hyperphagia in the albino rat. Yale J. Biol., 1943, *15*, 831.

8 Brooks, C. McC. and Lambert, E. F.: A study of the effect of limitation of food intake and the method of feeding on the rate of weight gain during hypothalamic obesity in the albino rat. Am. J. Physiol., 1946, *147*, 695.

9 Clark, George: The use of the Horsley-Clarke instrument on the rat. Science, 1939, *90*, 92.

10 Clark, George, Magoun, H. W., and Ranson, S. W.: Hypothalamic regulation of body temperature. J. Neurophysiol., 1939, *2*, 61.

11 Figge, F. H. J. and Salomon, Kurt: Porphyrin incrustations on pantothenic acid deficient rats. J. Laborat. Clin. M., 1942, *27*, 1495.

12 Fox, Clement A.: Certain basal telencephalic centers in the cat. J. Comp. Neur., 1940, *72*, 1.

13 Gershberg, H., Fry, E. G., Brobeck, J. R., and Long, C. N. H.: The rôle of epinephrine in the secretion of adrenal cortex. Yale J. Biol., 1950, *23*, 35 (footnote).

14 Hetherington, A. W.: The relation of various hypothalamic lesions to adiposity and other phenomena in the rat. Am. J. Physiol., 1941, *133*, 326.

15 Hetherington, A. W.: Non-production of hypothalamic obesity in the rat by lesions rostral or dorsal to the ventromedial hypothalamic nuclei. J. Comp. Neur., 1944, *80*, 33.

16 Hetherington, A. W. and Ranson, S. W.: Hypothalamic lesions and adiposity in the rat. Anat. Rec., 1940, *78*, 149.

17 Hetherington, A. W. and Ranson, S. W.: The relation of various hypothalamic lesions to adiposity in the rat. J. Comp. Neur., 1942, *76*, 475.

18 Hetherington, A. W. and Ranson, S. W.: The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Physiol., 1942, *136*, 609.

19 Ingram, W. R., Barris, R. W., and Ranson, S. W.: Catalepsy—an experimental study. Arch. Neur. Psychiat., Chic., 1936, *35*, 1175.

20 Kennedy, G. C.: Hypothalamic control of food intake in rat. Proc. R. Soc. Lond., 1950, *137*, 535.

21 Krieg, W. J. S.: The hypothalamus of the albino rat. J. Comp. Neurol., 1932, *55*, 19.

22 Ranson, S. W.: Somnolence caused by hypothalamic lesions in monkeys. Arch. Neur. Psychiat., Chic., 1939, *41*, 1.

23 Ranson, S. W.: Regulation of body temperature. Res. Pub. Ass. nerv. ment. Dis., 1939, *20*, 342.

24 Ranson, S. W., Fisher, Charles, and Ingram, W. R.: Adiposity and diabetes mellitus in a monkey with hypothalamic lesions. Endocrinology, 1938, *23*, 175.

25 Ruch, T. C., Patton, H. D., and Brobeck, J. R.: Hyperphagia and adiposity in relation to disturbances of taste. Fed. Proc., Balt., 1942, *1*, 76.

26 Wheatley, M. D.: Hypothalamus and affective behavior in cats. Arch. Neur. Psychiat., Chic., 1944, *52*, 296.

HOMOLOGOUS AND HETEROLOGOUS TRANSPLANTATION OF PANCREATIC TISSUE IN NORMAL AND DIABETIC MICE*

HENRY BROWNING AND PHILIP RESNIK

Greene[8] showed that embryonic tissues would survive, grow, differentiate, and organize after transfer to the anterior chamber of the eye. Such tissues often persisted in a healthy state for indefinite periods even in hosts of alien species. Liver and endocrine tissues, however, did not survive transplantations. Greene[10] later found that gonads could be successfully transplanted and showed that embryonic tissue would usually survive when transferred to a subcutaneous site.[9]

Browning[3] extended the above observations in regard to transplants of embryonic, neonatal, and adult tissues in the mouse. Embryonic tissues grew over a well-defined period after transfer to the same or alien strains of mice. The growth phase was followed by indefinite survival when transfers were made to the same strain and by regression and absorption in alien strains. Neonatal tissues usually did not grow but persisted indefinitely in the same strain and were absorbed in alien strains. Adult tissues never grew and might persist or be absorbed in the same strain; they were always absorbed in alien strains. In all cases except that of adult tissue, growth or survival was accompanied by differentiation and organization of the transplanted tissues.

The tissues transplanted which have or are said to have endocrine function included thymus, liver, and placenta. Thymus did not differ in behavior from other tissues in general; neither liver nor placenta survived transfer even to hosts of the same strain as that of origin.

The present report concerns the transplantation of pancreas both in normal and diabetic hosts. As in previous reports,[2,3] the term homologous has been applied to transfers between mice of the same inbred strain and the term heterologous to transfers between mice of different inbred strains.

Material and Methods

Embryonic pancreatic tissue was obtained from fetuses removed from mice on the sixteenth day after mating, and newborn tissue from mice born on the twentieth day after mating. These and adult pancreatic tissue were derived from inbred mice of the C_3H, dba, or C_{57} brown strains, and were implanted homologously or heterologously

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into mice of one of the appropriate three strains. The recipient mice were of either sex but always between three and four months of age.

The fate of different developmental stages of pancreatic tissue in homologous and heterologous transfer was examined in the anterior chamber of the eye. The technique of intra-ocular transplantation has been described previously.[2, 3] The entire pancreas of sixteen-day embryos was divided into four to six fragments, and each fragment was used as a separate transplant. Newborn and adult pancreases were divided into fragments of size similar to those of embryonic pancreas. For each developmental stage, fragments were transferred to the eyes of seven to ten homologous hosts and to the same number of heterologous hosts. Both eyes of each host were used and one fragment of pancreatic tissue placed in each anterior chamber. The procedure was repeated three times for each developmental stage using pancreatic tissue from independent sources.

Intra-ocular transplants were examined on the second, seventh, fourteenth, and twenty-first days and at the end of one, two, and three months. Except on the second day, representative animals were killed at the same intervals and their transplants used for histological study. Whole embryos at the thirteenth and sixteenth days of gestation and the pancreases of newborn, three-day post-natal, five-day post-natal, seven-day post-natal, and adult mice were also studied histologically. Pancreatic differentiation was examined by the staining methods of Bensley[1] and Gomori[6] both in this and in other histological material.

The fate and physiological function of embryonic and neonatal pancreatic tissue in diabetic hosts was also examined in the intra-ocular, subcutaneous, and subcapsular splenic sites. The hosts were always homologous and ten to fifteen recipients were used in each series. When the intra-ocular site was employed, either half or a whole sixteen-day embryonic pancreas was transplanted to each eye. The anterior chamber was entirely filled by a whole pancreas at this developmental stage. When the subcutaneous or subcapsular splenic sites were used, two or more sixteen-day embryonic or newborn pancreases were transferred to each host. Such transplants were preserved for histological study, together with the pancreas of the host, either at the death of the host or at the termination of the experiment.

Diabetic mice were produced by the intraperitoneal administration of alloxan in 1% aqueous solution, following a period of forty-eight hours' starvation. In mice of the dba strain a dosage of 175 mg./kg. produced a moderate to severe glycosuria (plus two to complete reduction in the Benedict qualitative test) in approximately seventy-five per cent of the animals. Mice of the C_{57} brown strain were completely refractory to alloxan, no gross symptoms appearing from the above dosage. A higher dosage or several repetitions of this dosage resulted in death without glycosuria. Consequently, dba mice were used exclusively in physiological function experiments.

Individual urine collections for testing of glycosuria by Benedict's qualitative reagent were made by placing each animal in a half-liter filter funnel. The tail of each mouse was taped to the funnel rim so as to restrict the animal and prevent adsorption of urine on the tail. Fine wire mesh at the exit of the funnel excluded feces from the urine draining from it. All food (laboratory chow) was withdrawn from the animals twenty-four hours before testing and replaced two hours before testing. Immediately prior to placement in the funnels each mouse received 1 ml. of water intraperitoneally. This procedure ensured within the subsequent two hours the excretion by each animal of urine sufficient for testing.

Results

A. Normal development of the pancreas in the mouse. In mice of each of the three inbred strains used the pancreas was distinguishable histologically on the thirteenth day of embryonic life. The anlage consisted of a number of

branching tubules, the lumina of which were lined with a low columnar epithelium. The anlage was not discernible in gross dissections.

By the sixteenth day of embryonic life the pancreas was well defined in gross dissections. Histological study showed that alveolar formations had developed and the lumina were almost completely obliterated by the swollen cells (Fig. 1). The newborn pancreas differed from this stage only in the increased number of alveoli.

TABLE 1

BIOLOGICAL BEHAVIOR OF INTRA-OCULAR TRANSPLANTS OF EMBRYONIC, NEWBORN AND ADULT PANCREATIC TISSUES OF MICE IN HOMOLOGOUS AND HETEROLOGOUS HOST STRAINS

	Homotransplants			*Heterotransplants*		
Stage of donor	*General development of transplants*	*No. with organization/ total no.*	*No. with organization and growth/ total no.*	*General development of transplants*	*No. with organization/ total no.*	*No. with organization and growth/ total no.*
Embryo	Growth up to 3 x original area; indefinite persistence	10/11 13/15 14/14	10/11 10/15 14/14	Rare growth; regression and absorption after 3rd week	10/15 12/16 10/13	3/15 3/16 2/13
Newborn	Rare growth; indefinite persistence	12/14 12/16 13/15	2/14 0/16 2/15	Very rare growth; regression & absorption after 3rd week	8/14 9/16 8/13	1/14 1/16 0/13
Adult	No growth; indefinite persistence of most	0/14 0/14 0/14	0/14 0/14 0/14	No growth; absorption from time of transfer	0/14 0/16 0/15	0/14 0/16 0/15

Further increase in the number of alveoli occurred during the first week of post-natal life. Between the first and third days the islets of Langerhans appeared (Fig. 2), but only beta cells were recognizable. The islets were distinguishable grossly as small opaque foci scattered in the semitranslucent alveolar tissue. Adult pancreas differed only in size and in the presence of alpha cells in the islets.

B. Development of intra-ocular transplants of pancreas. The general pattern of development of intra-ocular transplants of pancreas from sixteen-day embryonic, newborn, and adult mice in untreated homologous and heterologous hosts is shown in Table 1. In addition the number of transplants in each series that showed grossly visible organization, with or

without actual increase in size, and the number that showed organization with actual growth are given.

The majority of transplants of embryonic pancreas in homologous hosts grew to one and one-half to three times their original surface area. This surface extension was accompanied by proportionate volumetric increase which was not measurable. Vascularization was apparent in the transplants on the second or third day after transfer, and growth began immediately after, reaching completion by the twenty-first day. Thereafter the transplants persisted in an unchanged state for as long as the hosts were maintained. Small cysts often appeared in the transplants at the end of the first week of intra-ocular life; at the same time scattered opacities also became visible. The cysts might or might not increase in size during the rest of the growth period, while the opacities always enlarged until cessation of growth at the end of the third week. These opacities closely resembled those seen in the whole pancreases of mice more than three days old where they represented the islets of Langerhans. Fatty tissue was also developed during the latter half of the growth period.

Thirty-four of the forty transplants of embryonic pancreas showed the above pattern of growth and development. Three more transplants showed the same developmental pattern of vascularization, cysts, opacities, and fat without actual size increase. The remaining three transplants began to be absorbed from the time of transfer.

Homologous transplants of newborn pancreas usually showed development of vascularization, cysts, opacities, and fat without growth of the whole transplant. However, out of the thirty-seven fragments among forty-five that did develop, four also showed growth to approximately twice the original surface area. The eight transplants that showed no development began to be absorbed from the time of transfer.

Homologous transplants of adult pancreas often became vascularized and persisted indefinitely. They did not show any developmental changes involving cysts, opacities, or fat. Occasional opacities were visible if the transplanted fragment had contained islet tissue at the time of transfer. These opacities did not change. Not only was there no growth of any transplant as a whole, but those that persisted became reduced to thin sheets of tissue without proportional depth, quite unlike transplants of embryonic or newborn tissue. Out of forty-two fragments transferred, twenty-six showed the above course; the remaining sixteen began to be absorbed from the time of transfer.

Heterologous transplants of embryonic, newborn, and adult pancreas showed behavior generally similar to corresponding homotransplants. However, growth of the transplants as a whole was much less frequent and there was no indefinite persistence. In the case of embryonic pancreas only eight out of forty-four transplants grew, although thirty-two showed development of vascularization, cysts, opacities, and fat in a manner identi-

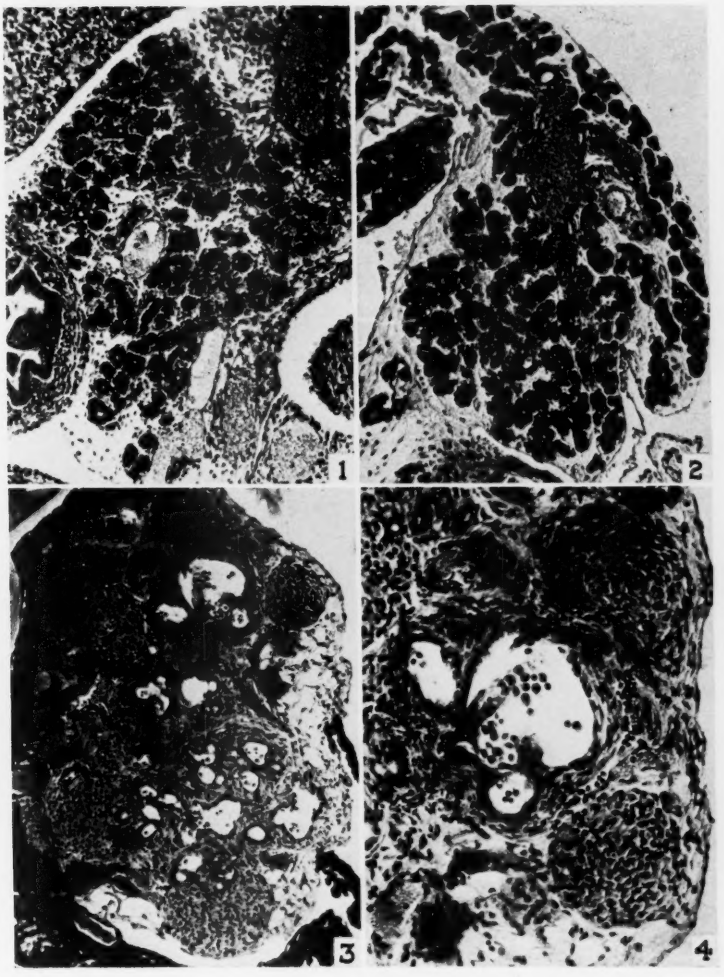

FIG. 1. Pancreas in 16-day embryonic dba mouse (x155).

FIG. 2. Pancreas of dba mouse on the third post-natal day (x155).

FIG. 3. Intra-ocular homologous transplant of 16-day embryonic pancreas after one month in a normal host (x155).

FIG. 4. Intra-ocular transplant of 16-day embryonic pancreas after one month in a normal host (x310).

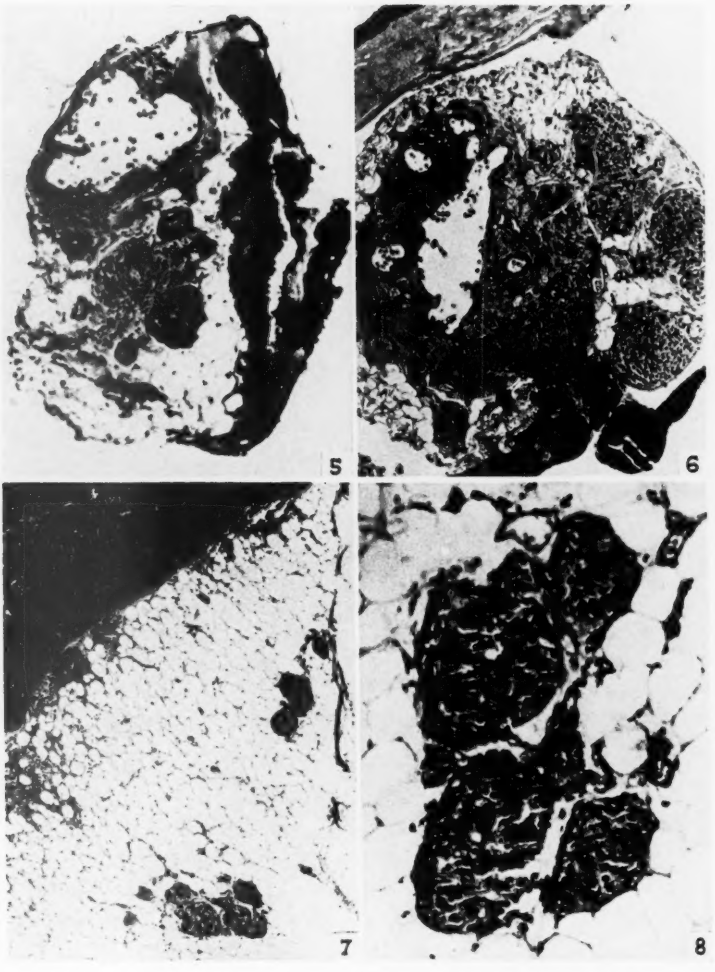

FIG. 5. Intra-ocular homologous transplant of newborn pancreas after two months in a normal host (x155).

FIG. 6. Intra-ocular homologous transplant of 16-day embryonic pancreas after one month in a diabetic host (x155).

FIG. 7. Part of subcutaneous homologous transplant of four newborn pancreases (with adjacent lymphnode) after three months in a diabetic host (x55).

FIG. 8. Part of subcutaneous homologous transplant of four newborn pancreases after three months in a diabetic host (x310).

cal to homotransplants. The remaining twelve transplants began to be absorbed from the time of transfer. Following the developmental period, between the end of the third and fourth weeks of intra-ocular life, all surviving heterotransplants began to regress. Vascularity and general translucence of the tissues were lost. The fragments diminished in size and were completely absorbed by the end of the fifth or sixth week.

In the case of heterotransplants of newborn pancreas, two out of forty-three showed over-all growth, and twenty-five underwent developmental changes, while the remaining eighteen began to be absorbed from the time of transfer. The developmental changes involved vascularity, cysts, opacities, and fat and occurred over the first three weeks of intra-ocular life, as did similar changes in other series of transplants. At the end of this time the newborn transplants regressed and were absorbed in the same manner as heterotransplants of embryonic pancreas. Heterologous transplants of adult pancreas began to be absorbed from the time of transfer and never showed any signs of development with or without growth.

Histologically the surviving transplants of embryonic or newborn pancreas in any series were found to consist of connective tissue with fat cells, vesicles of varying size and highly cellular islands (Figs. 3, 4, and 5). These islands were somewhat less numerous in transplants of newborn tissue. The vesicles were those seen grossly as cysts; they were lined with a cuboidal epithelium and were the only representative of alveolar tissue. The islands were exceptionally well vascularized, consisting of cells with very basophilic granular cytoplasm and round nuclei. Special stains demonstrated that they represented the beta cells of the islets of Langerhans. No other cell type occurred in these islands.

Histologically, homologous transplants of adult pancreas showed thin sheets of fat cells and fibroblasts. Islets occurred only if they had been present in the transplanted fragment.

In summary, homologous transplants of embryonic, newborn, and adult pancreas usually showed indefinite survival often accompanied by developmental changes and sometimes by growth of the transplant as a whole. The capacity for such survival, and especially for accompanying growth, diminished with increasing age of the donor of the transferred tissue. The developmental changes, absent in the case of adult transplants, involved good differentiation and organization of islet tissue and poor representation of the alveolar components of the normal gland. In heterologous transplants a limited survival accompanied by developmental changes with or without growth occurred but was less frequent. The capacity for such survival also diminished with increasing age of the donor. Regression and absorption always ended the course of heterotransplants.

C. Transplants of pancreas in diabetic mice. Seven groups of twelve to eighteen dba mice were treated with alloxan. The individual urines were tested after six to eight days when the majority of the animals gave a two

TABLE 2

RANGE OF INDIVIDUAL GLYCOSURIA IN DIABETIC MICE WITH AND WITHOUT TRANSPLANTS OF EMBRYONIC AND NEWBORN PANCREAS

Site and type of transplants	*Glycosuria before and after alloxan administration (and pancreatic transplantation)*									*No. of mice surviving on 90th day*
	No. of mice	*7 days before*	*6-8 days after*	*14 days after*	*21 days after*	*30 days after*	*45 days after*	*60 days after*	*90 days after*	
A. None	22	0 — +	++ — CR*	+++ — CR	++++ — CR	most dead	all dead			0
B. One, embryonic intra-ocular	21	0 — +	++ — ++++	+++ — CR	++++ — CR	most dead	all dead			0
C. Two, embryonic intra-ocular	20	0 — +	++ — CR	++ — CR	++ — CR	++ — CR	most dead	most dead	most dead	2
D. Two, embryonic splenic	22	0 — +	+++ — CR	++++ — CR	++++ — CR	all dead				0
E. Two, embryonic subcutaneous	20	0 — +	++ — CR	+++ — CR	+++ — CR	+++ — CR	most dead	most dead	most dead	2
F. Two, newborn subcutaneous	26	0	++ — CR	++ — CR	+++ — CR	+++ — CR	most dead	most dead	most dead	1
G. Four, newborn subcutaneous	38	0 — +	++ — CR	+++ — CR	+ — CR	0 — CR	0 — CR	0 — +++	0 — +++	25

* CR; complete reduction of Benedict's qualitative reagent.

plus to complete reduction reaction with Benedict's qualitative reagent. Those animals with lesser reactions were discarded. Similar tests before alloxanization had shown that almost all of the mice had a negative reaction; an occasional animal had a doubtful plus one reading.

Each of the seven groups received one of the following treatments: no transplant, one embryonic pancreas intra-ocularly, two embryonic pancreases intra-ocularly, two embryonic pancreases beneath the splenic capsule, two embryonic pancreases subcutaneously, two newborn pancreases subcutaneously, or four newborn pancreases subcutaneously. Each treatment was repeated once on a similar but independent group of alloxanized mice, except for that involving four newborn pancreases which was repeated twice. The transplantations were made on the second day after the initial post-alloxan testing, i.e., eight to ten days after alloxanization.

All surviving animals were retested for glycosuria on the fourteenth, twenty-first, thirtieth, forty-fifth, sixtieth, and ninetieth days after alloxanization. Table 2 shows the range of glycosuria for the two or three groups receiving each treatment.

The mice of the groups receiving no transplants, one embryonic pancreas intra-ocularly or two embryonic pancreases beneath the splenic capsule showed an identical course (A, B, and D, Table 2). The glycosuria, if not maximal at the first post-alloxan test, gave a four plus or complete reduction reaction by the third test, i.e., twenty-one days after alloxanization. By the thirtieth day all animals were dead or extremely emaciated and obviously moribund. None survived beyond the thirty-fifth day.

A similar course obtained for most of the animals in the groups that received two embryonic pancreases intra-ocularly or subcutaneously or two newborn pancreases subcutaneously (C, E, and F, Tables 2 and 3). Most succumbed before the forty-fifth day. However, seven out of sixty-six (9%) animals showed a glycosuria that remained constant at a three plus reaction or diminished from an early three plus to a two plus. Two died on the 48th and 50th days respectively, and five survived until sacrificed on the ninetieth day.

A similar course also obtained for thirteen out of thirty-eight animals that received four newborn pancreases subcutaneously (G, Table 2). All died by or shortly after the forty-fifth day. However, twenty-five (66%) of the animals survived for three months with a glycosuria ultimately ranging from a negative to a plus three reaction. Table 3 shows the individual variations in glycosuria in these survivors until the ninetieth day when they were sacrificed. The usual course was an initial maximal glycosuria that slowly fell, or an initial submaximal glycosuria that rose and then fell, or that fell without a rise.

The gross behavior and histological development of intra-ocular transplants in diabetic hosts was identical with that in untreated animals (Fig. 6). Those transplants that were placed beneath the splenic capsule showed

no surviving pancreatic tissue. Those transplants made subcutaneously, whether of embryonic or newborn pancreas, showed differentiation of islet tissue identical with those in intra-ocular sites in diabetic or normal hosts, but alveolar components were absent (Figs. 7 and 8). No histological differences were seen between transplants from survivors at the ninetieth day and those from animals that died earlier. No islet tissue was found in the pancreases of any alloxanized hosts.

Discussion

Embryonic, newborn, and adult transplants. In general the behavior of embryonic, newborn, and adult pancreas paralleled that found for various

Table 3

Course of Glycosuria in Individual Mice Surviving on the Ninetieth Day after Alloxan Administration with Subsequent Transplants of Four Newborn Pancreases in a Subcutaneous Site

Number of mice with	*Glycosuria before and after alloxan administration (and pancreatic transplantation)*							
same course of glycosuria	*7 days before*	*6-8 days after*	*14 days after*	*21 days after*	*30 days after*	*45 days after*	*60 days after*	*90 days after*
2	+	++++	CR	++++	+++	+++	+++	+++
2	+	++++	++++	+++	+++	++	+++	++
3	0	CR	CR	++++	+++	+++	++	++
2	0	CR	++++	++++	++	+++	++	++
4	0	+++	CR	+++	+++	++	++	++
3	0	++++	+++	++	0	+	0	+
4	0	+++	++++	++++	++	++	0	0
3	0	CR	++++	++	++	0	0	0
2	0	+++	+++	+++	++	0	0	0

non-endocrine tissues.[8] Embryonic tissues grew and differentiated, newborn tissues differentiated without growth, and adult tissues showed no developmental activity. The immature tissues persisted indefinitely in homologous transfer and regressed in heterologous hosts. Adult tissues might persist in homologous and were always absorbed in heterologous transplantation. In two respects pancreatic tissue differed from the typical case. Firstly, acinar components showed little development although tissues with similar histology, such as submaxillary salivary gland, gave good development with very numerous but somewhat cystic acini.[3] Further, alpha cells were absent from the differentiated islets. Simard[10] found that the islets in autogenous grafts of pancreas in the dog consisted almost entirely of beta cells. Secondly, a few of the transplants of newborn pancreas showed growth. Growth of other newborn tissues was rare, only skin and stomach exhibiting

it. Possibly there is a correlation between the ability of newborn tissues to grow on transfer and the relative or absolute incompleteness of differentiation and organization at birth. This growth of the entire newborn pancreas is even more striking when the lack of development of acinar tissues, which form the bulk of the transplanted fragment, is considered. Such growth was not correlated with cyst formation.

The absence of indefinite persistence of embryonic pancreas in heterologous transfer was also consistent with previous work on other tissues, but inconsistent with the results of Greene[8, 9] who found little difference between the developmental course of transplants of rabbit, mouse, and human embryonic tissues in homologous and heterologous hosts. Adequate explanation for this inconsistency is lacking. Both eyes were used for transplants in each animal here and in previous work, whereas Greene used but one. Heterologous transplants made by the present author were between different inbred strains while those of Greene were between different species. It is possible that inbred strains of one species are more sharply distinguished in regard to tissue compatibility than two non-inbred species.

Development of embryonic and newborn grafts of pancreas in homologous hosts was identical in the intra-ocular and subcutaneous sites, which confirms the work of Greene,[8, 9] who found little difference between them for embryonic transplants. However, grafts made subcapsularly in the spleen did not persist; information on their growth and differentiation is lacking as they were not recovered from the hosts before the thirtieth day after alloxanization. The obvious difference between such a site and the intra-ocular and subcutaneous is that venous drainage would be into the hepatic portal rather than directly into the systemic circulation. However, the grafts would also be in contiguity with the reticulo-endothelial tissue of the host. Murphy and Sturm[14] found that transplants of tumor tissue were destroyed if accompanied by a fragment of autologous (but not homologous) spleen. Woodruff and Woodruff[19] made similar findings for thyroid tissue transplants associated with autologous and homologous spleen.

Production of diabetic mice. A permanent hyperglycemia or glycosuria was not produced in "Swiss" mice by intravenous (Waisbren)[17] or intraperitoneal (Ruangsiri)[15] alloxan at dosages of 100 mg./kg. and 300 mg./kg. respectively. In rats, Kass and Waisbren[12] used a subcutaneous dosage of 175 mg./kg. administered after 48 to 60 hours fasting. They obtained almost uniform susceptibility and a hyperglycemia that was sustained for at least three months. The same technique, except for the use of the intraperitoneal route, was used here with success in dba mice. The resultant glycosuria never disappeared spontaneously in control or experimental animals and tended to increase in severity over the first month. On the other hand, glycosuria was not produced by alloxan in mice of the C_{57} brown strain. The refractoriness of certain species such as the guinea-pig, duck, toad, and owl has been previously recorded.[12, 18]

The extent of histological damage to islet tissue by alloxan in various species is apparently variable. Findings have ranged from destruction of beta cells alone[7] to total elimination of all islets.[5] Duff,[4] however, stated that no islets of Langerhans remain in rabbits or rats after prolonged periods of alloxan diabetes. The present findings are in agreement with this statement for the dba mouse.

Effect of transplants on glycosuria. The general conclusion arising from attempts to replace islet tissue function of the host by an intra-ocular or subcutaneous transplant is that one or even two entire embryonic or newborn pancreases are insufficient to restore normal balance. This is not unreasonable when it is realized that, while such transplanted tissues differentiate and often grow, the ultimate growth is never comparable to that which would occur if the organ or tissue were left *in situ*, i.e., the transplants ceased to grow when differentiation was complete and never reached adult proportions. Further, while actual counts were not made, it was grossly obvious that the number of islets appearing in transplants of entire newborn or embryonic pancreases did not approach the number found in the adult gland.

Four newborn transplants of pancreas did effect a very definite action in reducing urine glucose towards normal values in most of the experimental series receiving this number of developing glands. The glycosuria produced by alloxan in dba mice was almost always severe, giving at least a plus three reaction by Benedict's test. There was a suggestion in the results of the series where two embryonic or newborn pancreases were transplanted that a mild degree of glycosuria might have been alleviated but that severe degrees were beyond the control capabilities of the limited islet tissue of the transplants. Attempts to produce a mild glycosuria by lower alloxan dosage failed. The action of the drug seemed to be of an all-or-none character. A mild glycosuria controlled by intra-ocular transplants would allow examination of the results of removal of the transplants with a certainty not possible with the subcutaneous site.

The reasons for the failure of four newborn pancreases transplanted subcutaneously to control glycosuria in thirty-four per cent of the animals in the series involved was obscure. In almost any series of intra-ocular transplants some fragments fail to develop and the proportion of such failures is higher when newborn tissues rather than embryonic are employed.[3] In the series of intra-ocular transplants of newborn pancreases (Table 1) in intact hosts eighteen per cent failed to develop. Such failure of some or all of the individual glands in each of the group of four transferred might account for the lack of observable effect on glycosuria. Further, the percentage of failures in subcutaneous transfers may have been higher than for intra-ocular transfers. Woodruff and Woodruff[19] found that subcutaneous transplants of adult thyroid tissue were more likely to survive if transferred from the anterior chamber of the eye rather than directly from the

donor. Recovery of the subcutaneous transplants from sacrificed animals was not always successful owing to their gross resemblance to the subcutaneous fatty tissue in which they were embedded. Consequently, some animals in the surviving and non-surviving groups did not provide histological material. On the other hand an appreciable proportion of non-survivors showed transplants identical histologically with those of the survivors.

The hypothesis of Halsted[11] that a deficiency of the corresponding tissue is essential for successful transplantation of endocrine glands is not upheld by the present results. The course of development of anterior chamber or subcutaneous transplants of embryonic and newborn pancreas was identical in normal and diabetic hosts.

It is probably significant that very immature tissues were transplanted. Both here and in earlier work[8] it has been shown that such tissues have much greater growth and survival potentialities than adult tissues. However, transplants of adult pancreas in a diabetic host were not made, and the lack of effect of host deficiency cannot be stated for such mature tissues.

Summary

1. Differentiated islet tissue appeared in the pancreas of the developing mouse between birth and the third day of post-natal life.

2. Embryonic and newborn pancreas, when transplanted intra-ocularly into animals of the same strain, showed differentiation of islets but acini were only represented by occasional cysts. The transplants persisted indefinitely. Growth, in the sense of actual increase in size, was only frequent in embryonic transplants. Adult pancreas, similarly transplanted, usually showed persistence without change.

3. Similar transplants of embryonic and newborn pancreas to animals of a different inbred strain showed similar differentiation. Growth, even of embryonic pancreas, was rare. Both tissues regressed after the first three weeks of intra-ocular life. Adult pancreas began to be absorbed from the time of transfer.

4. Intra-ocular and subcutaneous transplants of embryonic and newborn pancreas in mice of the same strain with an alloxan diabetes showed differentiation and growth similar to those seen in intra-ocular transplantation in untreated hosts. Transplants of embryonic pancreas placed beneath the capsule of the spleen failed to survive.

5. Intra-ocular or subcutaneous transplants of one or two embryonic or newborn pancreases had no effect on the course of the glycosuria in the majority of alloxanized hosts. Subcutaneous transplantation of four newborn pancreases gave reduction of glycosuria and an apparently indefinite survival in sixty-six per cent of hosts.

References

1 Bensley, R. R.: Studies on the pancreas of the guinea pig. Am. J. Anat., 1911, *12*, 297.

2 Browning, H. C.: Heterologous and homologous growth of transplants during the course of development of spontaneous mammary tumors in C_3H mice. J. Nat. Cancer Inst., 1948, *8*, 173.

3 Browning, H. C.: Homologous and heterologous growth of transplants of various tissues during the course of development in the mouse. Cancer, N. Y., 1949, *2*, 646.

4 Duff, G. L.: The pathology of the pancreas in experimental diabetes mellitus. Am. J. M. Sc., 1949, *210*, 381.

5 Dunn, J. S., Sheehan, H. L., and McLetchie, N. G. B.: Necrosis of islets of Langerhans produced experimentally. Lancet, 1943, *1*, 484.

6 Gomori, George: Studies on the cells of the pancreatic islets. Anat. Rec., 1939, *74*, 439.

7 Gomori, George and Goldner, M. G.: Production of diabetes mellitus in rats with alloxan. Proc. Soc. Exp. Biol., N. Y., 1943, *54*, 287.

8 Greene, H. S. N.: The heterologous transplantation of embryonic mammalian tissues. Cancer Res., 1943, *3*, 809.

9 Greene, H. S. N.: The production of carcinoma and sarcoma in transplanted embryonic tissues. Science, 1945, *101*, 644.

10 Greene, H. S. N.: The use of the mouse eye in transplantation experiments. Cancer Res., 1947, *7*, 491.

11 Halsted, W. S.: Auto- and isotransplantation, in dogs, of the parathyroid glandules. J. Exp. M., 1909, *11*, 175.

12 Kass, E. H. and Waisbren, B. A.: A method for consistent induction of chronic hyperglycemia with alloxan. Proc. Soc. Exp. Biol., N. Y., 1945, *60*, 303.

13 Lukens, F. D. W.: Alloxan diabetes. Physiol. Rev., 1948, *28*, 304.

14 Murphy, J. B. and Sturm, Ernest: Conditions determining the transplantability of tissues in the brain. J. Exp. M., 1923, *38*, 183.

15 Ruangsiri, Chanai: Changes in islets of Langerhans in living mice after alloxan administration. Anat. Rec., 1949, *105*, 399.

16 Simard, Louis-Charles: Étude histologique de pancréas greffés dans la paroi abdominale, chez le chien. (Complexes neuro-insulaires, ganglions nerveux, cellules insulaires.) Rev. canad. biol., 1945, *4*, 264.

17 Waisbren, B. A.: Alloxan diabetes in mice. Proc. Soc. Exp. Biol., N. Y., 1948, *67*, 154.

18 West, E. S. and Highet, D. M.: Resistance of guinea pigs to action of alloxan. Proc. Soc. Exp. Biol., N. Y., 1948, *68*, 60.

19 Woodruff, M. F. A. and Woodruff, H. G.: The transplantation of normal tissues; with special reference to auto- and homotransplants of thyroid and spleen in the anterior chamber of the eye, and subcutaneously, in guinea pigs. Philos. Tr. R. Soc. London, B No. 619, 1950, *234*, 559.

EFFECTS OF CARCINOGENS ON EMBRYONIC MOUSE TISSUE TRANSPLANTS, WITH SPECIAL REFERENCE TO LUNG*

J. SUMNER WOOD, JR.†

The present series of experiments was undertaken to study the neoplastic potentialities of embryonic tissue, under normal conditions and under influence of carcinogens and other chemicals. This work was the outgrowth of an interest in the problem of the origin of the malignant cell in the adult, and an investigation of the stages in development during which cells may become neoplastic.‡

MATERIALS

Mice four to six weeks of age of strains A, C, CBA, C_3H, and C_{57} black were used, chiefly because these strains are notably homogeneous. Embryonic tissues used for subcutaneous grafts were limited to tissues from strains A, C, and CBA. Six mice were placed in each cage, and all mice were maintained on stock laboratory chow ("Nurish-mix") and water *ad libitum*. The temperature of the animal rooms was constant at 72° F.

Embryonic lung, liver, and gut tissues were obtained from 15-20-day-old embryos and transplanted subcutaneously in the axillary region. Although the exact age of each litter was not determined, in many cases the gestation period was calculated by the vaginal plug method, and in some instances, the age was estimated by crown-to-rump measurements of the embryo.

Hysterectomy was performed after each pregnant mouse was killed by cervical vertebral dislocation. The uterus and embryos were placed immediately in a petri dish moistened with isotonic saline to minimize desiccation. Each fetus was removed separately from the uterine wall and decapitated immediately after rupture of the amniotic sac. The lungs were excised and kept moist with isotonic saline solution. From each embryo it was usually possible to obtain four small pieces of tissue for implantation immediately after removal. The same transplantation procedure was used from embryonic liver and gut tissues.

A small piece of tissue, about one cubic millimeter in size, was placed in a 13-gauge trocar and subsequently transplanted into the axillary region of the host. Most animals received two transplants, one on each side.

Homologous (to the same strain) and heterologous (to a different strain) transplants were made in each case. Transplants were made with untreated tissues and with tissues exposed to carcinogens.

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The effects on embryonic tissues of 20-methylcholanthrene and of the two azo dyes, namely, o-amino-azotoluene and m'-methyl-p-dimethyl-amino-azobenzene, were studied by implanting a single crystal of each compound in the middle of the graft prior to implantation. Urethane was administered by two methods. In one group, a single crystal was imbedded with the graft at the time of implantation; in a second group, a 0.08% or a 0.12% aqueous solution of urethane was supplied in the drinking water of the host after the embryonic lung transplant was accomplished. The 0.08% solution of urethane proved to be a relatively nontoxic dose for four- to five-week-old mice.

Tissues were fixed in 10% formalin and imbedded in paraffin. Representative sections, in many instances serial, were made of all blocks, and stained by the usual hematoxylin-eosin technique or Masson's trichrome method for connective tissue.

Results

Histologically, the embryonic lung tissue removed for implantation showed well-developed bronchi and few to many small bronchioles lined by cuboidal cells and separated from one another by compact mesenchyme. Occasionally, alveolar ducts were present.

After transplantation, dilatation of the alveoli occurred within a week of growth. Hemorrhage from surrounding host tissue was minimal at this time, and the few hemorrhagic areas showed large numbers of macrophages containing hemosiderin. During the early stages of growth, the implant was encapsulated by loose collagenous tissue. The host tissue showed lymphocytic infiltration, proliferation of fibroblasts, and edema.

As reported by Waddell,[18] the differentiation of the grafts exhibited two extremes: (i) In some cases, the alveoli were expanded and the bronchi and interstitial tissue well formed. The bronchial epithelium was ciliated and retained its normal histological appearance after more than a year. Cartilage surrounded the large bronchioles. The detection of persisting pseudo-stratified ciliated columnar epithelium positively identified the lung transplant. Differentiation similar to the "emphysematous condition" reported by Greene[7] was observed. (ii) On the other hand, some grafts showed fibrotic or lymphocytic infiltration, dilated bronchial lumina, and occasional metaplasia of the respiratory-type epithelium to the cuboidal or squamous types.

Numerous other grafts presented transitional conditions between the two extremes. Histologically, the alveolar epithelium of these grafts was composed of simple squamous cells. The alveolar lumina were filled with an eosinophilic material suggestive of a protein-containing fluid in which were seen amorphous debris, macrophages, and desquamated lining cells apparently degenerating. Transplants of embryonic lung that persisted for longer periods of time (ranging up to one year) showed an enlargement of the peribronchial lymph nodules.

The experiment designed to study the effect of urethane, administered orally, on the subsequent growth of homologously transplanted embryonic lung grafts showed that urethane in the amounts used had no detectable effect on the embryonic graft.

All of the strain A mice died or were sacrificed before any definite preliminary adenomatous changes in the adult host lungs could be detected. Three mice of the CBA strain treated for 71-80 days had small adenomas arising from a proliferation of septal cells. Similar early neoplastic lesions have been described by Mostofi and Larsen,[12]* Nettleship, Henshaw, and Meyers,[13] and Selbie and Thackray.[14] The majority of the mice fed urethane had significant interstitial pneumonia with lymphocytic and granulocytic infiltration.

One hundred and eighty-seven homologous transplants of embryonic lung were placed subcutaneously into 94 mice of strains A and CBA that

TABLE 1

EMBRYONIC LUNG GRAFTS IN THE SUBCUTANEOUS TISSUES OF URETHANE-FED AND CONTROL MICE OF THE A AND CBA STRAINS

Strain	*Treatment*	*Number of mice*	*Number of embryonic lung grafts*	*Number of tumors in embryonic lung grafts*	*Number of tumors in host grafts*
A	Urethane	62	124	0	0
CBA	Urethane	32	63	0	3 adenomas
		—	—	–	–
	Total	94	187	0	3
A	Controls	8	16	0	0
CBA	Controls	10	20	0	0
		—	—	–	–
	Total	18	36	0	0

were fed urethane (Table 1). Eighteen mice of these same strains (often litter mates of the urethane-fed animals) with 36 homologous transplants were used as controls, two transplants in each animal. None of the grafts showed neoplastic changes in either the control or the urethane-fed groups, while small adenomas appeared in three of the host lungs in the urethane-fed series.

Of 129 homologous grafts of embryonic lung, one single transplant showed a small early pulmonary adenoma after 183 days of growth (Table 2). Since this same animal bore an additional graft of embryonic lung containing a crystal of o-amino-azotoluene in the opposite axilla, it is possible that this adenoma originated from the action of the carcinogen rather than spontaneously.

* After this manuscript was presented for publication, the following article has appeared: Mostofi, F. K. and Larsen, C. D.: The histo-pathogenesis of pulmonary tumors induced in strain A mice by urethan. J. Nat. Cancer Inst., 1951, *11*, 118.

Cameron and Oakley[6] reported that hepatic cells from adult animals failed to survive implantation. Browning[5] failed to obtain viable embryonic liver transplants. In the present experiment, all embryonic liver grafts also failed to persist.

Pulmonary tumors arose in three embryonic lung transplants containing a methylcholanthrene crystal in three different mice. Two were small adenomas found when the animals were sacrificed 114 and 116 days after transplantation, while the third was sacrificed at 190 days. The third tumor (190 days) was morphologically a bronchial adenocarcinoma which grew rapidly in the host, failed to survive heterologous transplantation, but grew when transplanted autologously. The diagnosis of adenocarcinoma was justified on a morphological basis because of the compact glandular arrangement of the cells, the nuclear pleomorphism and hyperchromatism, and the presence of multinucleated cells. Pulmonary tumors also have been observed under similar conditions by other investigators (Greene[9] and Smith[16]).

Miller and Baumann[11] observed hepatomas in rats fed a diet to which m′-methyl-p-dimethyl-amino-azobenzene was added. In the present experiments, crystals of m′-methyl-p-dimethyl-amino-azobenzene were added to 10 embryonic lung grafts and one transplanted fragment of homologously implanted strain A adult lung. No tumors were noted in grafts surviving up to 254 days (Table 2).

Each of seven grafts of embryonic lung and each of two grafts of homologously transplanted strain A adult lung containing crystals of urethane produced no tumors in grafts surviving up to one year (Table 2). Other investigators have observed pulmonary adenomas in mice treated with urethane (Nettleship and Henshaw,[13] Larsen,[10] and Smith and Rous[15]), in some cases even after transplacental exposure.[10, 15]

Andervont and associates[1, 2, 3, 4] induced hepatomas, hemangioendotheliomas, and pulmonary tumors in mice of different strains by subcutaneous administration of o-amino-azotoluene. In the present experiment, 54 transplants treated with o-amino-azotoluene showed no neoplastic lesions (Table 2). The transplants were observed during a period extending up to 254 days after transplantation.

Discussion

The problem of the nature and the significance of the alveolar dilatation in embryonic transplants has confronted many investigators. The structure of the alveoli seen in embryonic lung grafts (Smith[17] and Waddell[18]) has been assumed by Waddell[18] to be the result of normal differentiation. Fetal rabbits decapitated *in utero* below the trachea showed alveolar dilatation (Jost and Policard[9]). In the experiments reported in the present paper, whenever embryonic gut, trachea, or esophagus was transplanted, the grafts frequently gave rise to cysts filled with an acidophilic fluid and often contained desquamated lining cells, cellular debris, and numerous macrophages

similar to the picture found in the alveolar lumina. As suggested by Jost, closure of the trachea may cause retention of serous material which may otherwise have entered the amniotic sac. In an expandable tissue such as

TABLE 2

SUMMARY OF OBSERVATIONS ON DIFFERENT EMBRYONIC AND ADULT TISSUES TRANSPLANTED SUBCUTANEOUSLY WITH OR WITHOUT ADDED CARCINOGEN

		No. of transplants		*No. transplants examined hist.*		
Treatment	*Tissue transplanted*	*Homol-ogous*	*Heterol-ogous*	*Homol-ogous*	*Heterol-ogous*	*No. tumors*
Untreated controls	Adult lung	9	..	2	..	0
	Emb. lung	93	11	47	1	1*
	Emb. gut	7	..	4	..	0
	Emb. liver	8	5	0	0	0
	Emb. skin, heart, and kidney	2	1	0	0	0
	Total	119	17	53	1	1
20-Methyl-cholan-threne	Emb. lung	3	..	3	..	3†
m'-Methyl-p-dimethyl-amino-azobenzene	Adult lung	1	..	0	..	0
	Emb. lung	10	..	0	..	0
	Total	11	..	0	..	0
Urethane	Adult lung	2	..	1	..	0
	Emb. lung	7	..	1	..	0
	Total	9	..	2	..	0
o-Amino-azotoluene	Emb. lung	26	4	10	0	0
	Emb. liver	9	5	0	0	0
	Emb. gut	9	..	5	..	0
	Emb. kidney	..	1	..	0	0
	Total	44	10	15	0	0

* Small adenoma.
† 2 small adenomas, 1 adenocarcinoma.

the lung, this fluid may easily cause pressure on the terminal bronchioles and thereby cause alveolar dilatation in a manner similar to the cyst formation seen in other transplants of esophagus, bronchus, or gut.

Adult mouse lung tissue is notoriously susceptible to the carcinogenic action of urethane.[13, 14] As may be seen in Table 2, the host lung developed

adenomas in three cases in which embryonic lung grafts remained unaffected when urethane was fed in the drinking water (no adenomas in 187 embryonic lung grafts in 94 mice to which urethane was administered orally). Larsen[10] and Smith and Rous[15] reported pulmonary adenomas in mice within the first two weeks of age when they had been exposed *in utero* to urethane administered orally or injected into the mother. In this respect, the capacity of transplanted fetal lung tissue to produce tumors in embryonic tissue exposed to urethane administered to the host orally after transplantation differed from that of intact lung tissue. None of 187 transplants so treated gave rise to tumors.

TABLE 3

SUMMARY OF ALL SUBCUTANEOUS TRANSPLANTS REPORTED IN EXPERIMENT

	No. of transplants	*No. of tumors*
Untreated tissue	172	1
Embryonic lung grafts in animals fed urethane	187	0
Grafts treated with:		
20-Methylcholanthrene	3	3
m′-Methyl-p-dimethyl-amino-azobenzene	11	0
o-Amino-azotoluene	54	0
Urethane	9	0
Total	436	4

Despite the small number of observations, the high incidence of pulmonary tumors arising in transplants of fetal lung containing crystals of methylcholanthrene confirms the experiments previously reported by Greene[8] and Smith.[16] The failure of lung tumors to appear in grafts containing crystals of o-amino-azotoluene, m′-methyl-p-dimethyl-amino-azobenzene, or urethane (Table 3) indicates (i) that the dosage was too low or mode of administration inadequate, or (ii) that these agents are not directly effective carcinogenic stimuli for embryonic lung, or (iii) that embryonic tissue transplanted in the adult host is not particularly sensitive to these chemicals.

SUMMARY

All of three transplants of embryonic lung tissue treated with 20-methylcholanthrene became neoplastic.

Transplants of various embryonic and adult tissues treated by inclusion of crystals of o-amino-azotoluene, m′-methyl-p-dimethyl-amino-azobenzene, or urethane failed to become tumorous. Urethane administered to the host failed to induce tumors in transplanted embryonic lung tissue.

Of 129 untreated homologous transplants of embryonic lung, one gave rise to a small pulmonary adenoma in the graft.

References

1 Andervont, H. B.: Pulmonary tumors in mice. IX. The induction of pulmonary tumors in strain A mice by injection of 2-amino-5-azotoluene or 3,4,5,6-dibenzcarbazole. Pub. Health Rep., Wash., 1939, *54*, 1529.

2 Andervont, H. B.: The susceptibility of five inbred strains of mice to liver changes induced by subcutaneous injection of 2-amino-5-azotoluene. Pub. Health Rep., Wash., 1939, *54*, 1986.

3 Andervont, H. B., Grady, H. L., and Edwards, J. E.: Induction of hepatic lesions, hepatomas, pulmonary tumors, and hemangioendotheliomas in mice with o-amino-azotoluene. J. Nat. Cancer Inst., 1942, *3*, 131.

4 Andervont, H. B. and Edwards, J. E.: Carcinogenic action of two azo compounds in mice. J. Nat. Cancer Inst., 1943, *3*, 349.

5 Browning, H. C.: Homologous and heterologous growth of transplants of various tissues during the course of development in the mouse. Cancer, N. Y., 1949, *2*, 646.

6 Cameron, G. R. and Oakley, C. L.: Transplantation of liver. J. Path. Bact., Lond., 1934, *38*, 17.

7 Greene, H. S. N.: The heterologous transplantation of embryonic mammalian tissues. Cancer Res., 1943, *3*, 809.

8 Greene, H. S. N.: The production of carcinoma and sarcoma in transplanted embryonic tissues. Science, 1945, *101*, 644.

9 Jost, A. and Policard, A.: Contribution expérimentale a l'étude du développement prénatal du poumon chez le lapin. Arch. anat. micr., Par., 1948, *37*, 323.

10 Larsen, C. D.: Pulmonary-tumor induction by transplacental exposure to urethan. J. Nat. Cancer Inst., 1947, *8*, 63.

11 Miller, E. C. and Baumann, C. A.: The carcinogenicity of certain azo dyes related to p-dimethylaminoazobenzene. Cancer Res., 1946, *6*, 289.

12 Mostofi, F. and Larsen, C. D.: Cytogenesis of pulmonary tumors induced by urethan. Cancer Res., 1949, *9*, 544.

13 Nettleship, A., Henshaw, P. S., and Meyers, H. L.: Induction of pulmonary tumors in mice with ethyl carbamate (urethane). J. Nat. Cancer Inst., 1943, *4*, 304.

14 Selbie, F. R. and Thackray, A. C.: Lung adenomas induced by urethane in CBA mice. Brit. J. Cancer, 1948, II, 380.

15 Smith, W. E. and Rous, P.: The neoplastic potentialities of mouse embryo tissue. IV. Lung adenomas in baby mice as result of prenatal exposure to urethane. J. Exp. M., 1948, *88*, 529.

16 Smith, W. E.: The neoplastic potentialities of mouse embryo tissues. V. The tumors elicited with methylcholanthrene from pulmonary epithelium. J. Exp. M., 1949, *91*, 87.

17 Smith, W. E.: The tissue transplant technic as a means of testing materials for carcinogenic action. Cancer Res., 1949, *9*, 712.

18 Waddell, W. R.: Organoid differentiation of the fetal lung. Arch. Path., Chic., 1949, *47*, 427.

EVENTS

PHARMACOLOGY SEMINAR

October 18, 1951

SOME ASPECTS OF THE APPLICATIONS OF CATIONIC EXCHANGE RESINS IN MEDICINE. By Evan W. McChesney, Senior Member Biology Division, Sterling-Winthrop Research Institute, Rensselaer, New York.

A recent and promising method for salt-depletion in the treatment of edema is the feeding of cationic exchange resins. These are compounds containing numerous acidic groups, which, in the presence of salts, may give up their hydrogen atoms and fix metallic ions. There are two principal types of cationic exchange resins, one a polymer of sulfonated vinyl and divinyl benzene, and the other a polymer of methacrylic acid and divinyl benzene. The former, a strong acid, gives up its hydrogen ions rapidly in exchange for metallic ions, whereas the latter, a weak carboxylic acid, reaches equilibrium in its exchange reaction only over a period of several days. The carboxylic acid polymer, however, has about twice the exchange capacity by weight. In spite of these differences, at physiological pH there is little difference in their behavior.

Some of the original enthusiasm for the use of resins in place of salt-free diets has waned. The amount of sodium absorbed by the resins in rats appears to be influenced by the level of sodium in the diet. On high intakes, only a portion of the intake is absorbed, whereas in salt-free diets, virtually no sodium is excreted via the resin. There is no adequate explanation for this phenomenon. The suggestion that the exchange takes place entirely in the stomach, before exposure to the salt-containing pancreatic secretions, does not hold, as the carboxylic polymers are inactive at the pH of the stomach.

Cationic exchange resins have affinities for ions other than sodium—in fact, the strength of affinity increases in this order: sodium, potassium, bivalent ions. Fecal analysis in rats indicates that some magnesium, very little calcium (probably due to the insoluble state of a large portion of the calcium in the gut), and an appreciable amount of potassium are removed by the resin. The problem of potassium depletion is successfully solved by using resins in which one mEq. of potassium per gram of resin has previously been substituted. Ammonium ion is also removed, but not amino nitrogen, thiamin, or riboflavin.

W. P. McN.

YALE MEDICAL SOCIETY

October 31, 1951

EXPERIENCES IN KOREA WITH MEDICAL CARE IN FORWARD AREAS. By Captain E. R. Hering, MC, U.S.N., Field Medical Service School, Camp Jejeune, North Carolina.

In November, 1950, after some fifty thousand Chinese troops attacked UN positions near the Chosen reservoir in Korea, forty medical officers handled six thousand casualties in six days. Four thousand of the men, of which two thousand were suffering from frostbite, walked to the rear in weather thirty degrees below zero. Such a military operation was possible because of the way in which the medical corps is organized. The fast moving chain of evacuation begins when an injured man is moved to a battalion aid station by a platoon aid man. After administration of plasma and/or whole

blood he is transferred to a collecting and clearing station for surgical procedures including attention to uncontrolled bleeding, damaged viscera, and chest wounds. Some miles to the rear he may be hospitalized or flown to distant general hospitals.

Civilian defense officials should profit from the vast military medical experiences in Korea. A realistic, well-organized system, centered around small, flexible installations rather than large, permanent hospitals will be of greatest value if our cities are attacked. Civilians must concentrate on casualty sorting, supply, purchasing, and transportation procedures as do the military. Certain aspects of public health and preventive medicine, including mass immunizations for tetanus, and the like, must not be neglected.

J. K. R.

PHARMACOLOGY SEMINAR

November 1, 1951

PHYSIOLOGICAL DISPOSITION OF DRUGS LABELLED WITH RADIOACTIVE CARBON. By Paul K. Smith, Ph.D., Professor and Chairman of the Department of Pharmacology, George Washington University School of Medicine, Washington, D. C.

Radioactive carbon, although it has a half-life of six thousand years, can be used safely in studying the mechanism of drug action because it is eliminated from the body within thirty days. Labelled salicylates were given to both experimental animals and humans. Chromatography and Craig's counter-current technique were used to isolate metabolites of the drugs. The metabolic products of salicylates separated by this technique were identical with those isolated previously by classical methods. The fates of purines and pyrimidines are being explored in a similar fashion.

J. P. G.

NATIONAL ACADEMY OF SCIENCES

Public Lecture

November 5, 1951

THE MICROSTRUCTURE OF LIVING CELLS AND THEIR CONSTITUENTS. By Francis O. Schmitt, Massachusetts Institute of Technology, Cambridge, Massachusetts.

By application of special optical methods, such as polarized light microscopy, it is now possible to infer in the living cell the presence of structures of several hundred Å in size. With the electron microscope the mitochondrial membrane can be seen, while studies of its birefringence have led to an elucidation of its molecular structure. Chemical isolation studies implicate the mitochondria in the synthesis of ATP and show that the spatial relationships of the mitochondrial enzyme molecules are important in determining their characteristic activity.

Application of these techniques has led to many advances in the study of chromosomes, microsomes, and other cellular constituents. Electron microscopic shadowgraph techniques reveal much of crystalline structure, and X-ray diffraction patterns expose more and more of the intra-molecular configurations of proteins. Protein fractionation and electrophoretic studies are being used to investigate proteins and their activity in such cells as the cleaving sea urchin egg and the squid giant axon. Important discoveries will undoubtedly come from the increasing use of these last named techniques.

Collagen fibers and a non-naturally occurring fiber with a longer periodicity have been reproduced *in vitro*. The technique involves dissolving collagen fibers in acetic acid, passing the solution through a sintered glass filter and crystallizing the water-clear filtrate. This results in the deposition of very small, apparently unorganized fibers as seen through the electron microscope. Addition to the filtrate of a given quantity of salts, or a small amount of glycoprotein with subsequent crystallization, yields highly organized collagen fibers with the same periodicity as that seen in nature (i.e., 640 Å). Addition of larger amounts of glycoprotein results in the formation of the long-spaced fibers, which have a characteristic periodicity of their own. These observations suggest that the *in vitro* production of other cellular structures and, possibly, of entire cells may soon be accomplished.

J. M. Q.

PHARMACOLOGY SEMINAR

November 6, 1951

Newer Methods of Collecting Blood for Transfusion. By Edwin J. Cohn, Chairman, Division of Medical Sciences, Harvard University.

Processes for the fractionation of plasma have been considerably improved since the last war, so that the various protein fractions may now be isolated with very little change from the natural state. By the older methods, for example, concentrations of ethanol up to 18% and pH as low as 5 were required for the precipitation of albumin. The use of low concentrations of zinc, and in some cases cadmium and lead, significantly lowers the concentration of ethanol required and shifts the point of maximum insolubility toward the physiological range of pH. The metallo-protein complexes so formed are dissociable, and subsequent to separation the ions may be removed with exchange resins.

A machine for immediate fractionation of donated blood was demonstrated. Anticoagulants are not used. The blood is led directly from the vein through a column of exchange resin to remove platelets and calcium. It then passes into a continuous centrifuge, that separates red cells, white cells, and (quantitatively) plasma. Platelets may be eluted from the column, and the plasma may be treated directly for protein separation.

W. P. McN.

PHARMACOLOGY SEMINAR

November 8, 1951

The Action of Fibrillatory and Antifibrillatory Drugs on Cardiac Excitability. By C. McC. Brooks, Professor and Chairman of the Department of Physiology and Pharmacology, State University of New York College of Medicine, New York City.

The practical and theoretical implications of the recovery phase of excitation have long interested students of the cardiac cycle. The speaker and his co-workers used the open chest of a cat or dog. The heart was driven at a constant rate by an artificial pace-maker, time being plotted as abscissae against thresholds determined by a testing stimulator. The resulting graphs reveal that recovery is not a process which continues at a constant rate of declining threshold, but is represented by a line which dips and rises during the general descent. Stimuli above the threshold ordinarily produce extrasystoles, but result in fibrillation at the dip phase. Extrasystoles may be induced even after a long latent period when the tissue is stimulated early in the T wave of the electrocardiogram. Further

study with this preparation shows that a rise in temperature or heart rate shortens recovery by influence on the absolute rather than the relative refractory period. The auricular effects of vagal stimulation are found to be: increased rate of conduction, shortened recovery period, and increased susceptibility to fibrillation.

Since these studies suggested differences in the chemical aspects of phases of recovery as well as in susceptibility to fibrillation, investigation of drug effects was undertaken. Quinidine does not, as one might expect, abolish the dips (corresponding to periods of maximum vulnerability to fibrillation) in the curve, but it does prolong both the relative and absolute refractory periods. Amarin (a drug similar to chloroform in its ability to render the heart vulnerable to epinephrine fibrillation), surprisingly enough, resembles quinidine with reference to electrical stimulation in that it increases the absolute refractory period. Such experiments suggest that electrical stimulation may operate by a different mechanism than chemical, and hence, "it may be possible to fibrillate the heart by more than one dysfunction." Other hypotheses were suggested, however: (1) that slowed conduction of one drug may allow two waves to exist on the heart at once, should a stimulating drug be added, or (2) that with augmentation of the origin of beats by one drug and shortening of refractory period by another, normal conduction might now be too slow to prevent the simultaneous existence of two waves. Thus "the normal processes are out of phase" permitting "the disorientation which is fibrillation."

O. L. K.

BOOK REVIEWS

TIME'S ARROW AND EVOLUTION. By Harold F. Blum. Princeton, The Princeton University Press, 1951. xi + 222 pp. $4.00.

Dr. Blum's main thesis in this small volume is that from the origin of life, evolution has proceeded necessarily along certain channels, predetermined from without by the physical nature of the inorganic earth and by thermodynamic considerations from within. His interpretation of the free energy changes in biochemical reactions is particularly lucid. The exposition draws freely from the disciplines of cosmogony, physical and photochemistry, to name a few. There is no denial of the importance of natural selection as an evolutionary force, but rather a setting of limits within which this force may operate.

The title suggests an attempt at explanation of the mechanism of evolution on the basis of the second law of thermodynamics. No such explanation is presented. However, the ghost of "living systems being a contradiction of the second law" is forcefully laid by emphasizing the requirement of a closed system for measurements. The living organism is not a closed system, nor is the earth which it inhabits. In order for life to constitute a violation of this law, it must be proven that the local decrease in entropy in the living thing is not compensated for by greater increases elsewhere in the same system.

The text in general is clearly written and self-explanatory, demanding little technical training of the reader. Particularly stimulating are the chapters entitled, "The Fitness of the Environment" and "The Origin of Life."

W. P. McN.

MYCOTROPHY IN PLANTS. By A. P. Kelley. Waltham, Mass., Chronica Botanica Company, 1950. xiv + 223 pp. $4.50.

This book takes the form of twelve lectures on the subject of the symbiotic associations of fungi and the various groups of terrestrial plants. It is actually a review article on the topic, covering the literature from the beginning of the nineteenth century to about 1940. It is not surprising to find that most of the book is devoted to the subject of mycorrhizae, or root-fungus associations, for this aspect of mycotrophy has received a great deal of attention from plant scientists because of its importance in forestry as well as physiology.

The lectures (or chapters) include discussions of the rise of the study of mycotrophy, the taxonomic ranges of both the host plants and the fungal endophytes, the wide geographical occurrence of mycotrophy, the relation of mycotrophic plants to their environment, the morphology of mycorrhizae, and the theories of the physiological significance of mycorrhizae. The author points out that little experimental work has been done on mycorrhizal physiology; in the preface he makes reference to a few recent (1949) papers which describe an approach to the problem through the use of radioactive elements as tracers. Since the publication of the book, several more such papers have appeared and may mark the beginning of a serious attempt to solve the problems involved. If this is the case, one may hope that a new chapter will soon be necessary.

Obviously the book has not been designed as an introduction to the subject of mycotrophy; the author assumes that the reader is familiar with

botanical terminology and with about as much of the subject matter as may be gotten from a text on mycology or plant physiology which stresses the topic. As a summary and discussion of its special subject this book serves a valuable function, but it is certainly not for casual reading.

One notices the stylistic limitations so common to reviews. There is scarcely a paragraph without a half dozen references to the twenty-five page bibliography. There are the inevitable discontinuities in going from one topic to another, and in the descriptions of the researches of the various workers. Yet whatever its readability, botanists will be glad to have this book on their reference shelves.

R. R. L. G.

BIOLOGICAL FOUNDATIONS OF HEALTH EDUCATION. Proceedings of the Eastern States Health Education Conference, April 1-2, 1948. New York, Columbia University Press, 1950. v + 169 pp. $2.50.

This volume is comprised of fourteen papers by outstanding men and women in the fields of nutrition, psychiatry, gerontology, and epidemiology. The papers are oriented around the theses that the lag between knowledge and application is long, as is that between knowledge and understanding. As one might expect, the articles are devoted to the explanation and development of the significance and applications of scientific knowledge and discoveries. The important contributions of the biological and medical sciences to health and health education are stressed and personally interpreted by those in each field.

The breadth of the philosophical implications is evident from the first essay, C. E. Turner's "Health education, yesterday and today," through "Orientation in geronotology" by E. J. Stieglitz, to "The social philosophy of health" by E. L. Bortz. This volume is of special value since it welds together the work of the conference.

J. K. R.

JAMES LIND, FOUNDER OF NAUTICAL MEDICINE. By Louis H. Roddis. New York, Henry Schuman, Inc., 1950. xi + 177 pp. $3.00.

After reading *James Lind* by Captain Roddis, one agrees with the publisher that "Life at sea in the days of the old line-of-battleships and frigates in the time when Captain Hornblower was a midshipman is well described by the author of this book." As a history of naval medicine and hygiene in the eighteenth century this book is unquestionably superior.

Roddis' presentation of James Lind's life includes a listing of his published works and a brief chronology of his life and times. Lind, justly named the founder of nautical medicine, was among the first to establish the value of citrus fruit juices for the prevention and treatment of scurvey and the use of cinchona bark in the treatment of malaria. He was most active in instituting various hygienic and public health measures which helped check ravages of typhus and other diseases abroad ship. He wrote *A treatise of the scurvy* (1753), *An essay on the most effectual means of preserving the health of seamen in the Royal Navy* (1757), and *An essay on diseases incidental to Europeans in hot climates* (1768), all of which are considered classics in the field of medicine. Lind was also the first to devise a practicable method for the shipboard distillation of fresh water from sea water.

It is unfortunate that Roddis presents Lind as a personage and tells us so little of the person, or rather of the personality, with the drives and motivations which made for such a wide spectrum of researches and activities. But despite the rather too academic and impersonal handling, the story of James Lind offers interesting and profitable reading.

J. M. Q.

INTRODUCTION TO MOTHERHOOD. By Grantly Dick Read. New York, Harper and Brothers, 1950. xviii + 104 pp. $1.75.

This little volume, "a basic primer for the woman having her first baby," will be welcomed by many for its conversational tone and forthrightness. It is written clearly, simply, and briefly, and makes no attempt to cover the complex subject of childbirth as Dr. Read did in *Childbirth Without Fear*. Labor, personal hygiene, and postnatal care occupy the major part of the volume. The concluding pages are devoted to an explanation of antenatal exercises, labor and delivery positions, and relaxation. This section is accompanied by a number of simple sketches.

"The object of this small book is to set out in simple words the method by which natural motherhood, in its widest sense, may be attained and maintained." This first sentence of his introduction nicely sums up Dr. Read's latest book.

J. K. R.

HORMONE ASSAY. Edited by C. W. Emmens. New York, Academic Press Inc., 1950. 356 pp.

This book fulfills a need that has been evident for some time—that of collecting in one place a description of the accepted procedures for conducting hormone assays. From this point of view alone it should come into widespread use, since it covers the accepted methods for determining the activity of a large number of hormones, viz. insulin, parathyroid hormone, adrenalin, pituitary hormones (posterior lobe, melanophore expanding hormone, ACTH, TSH, lactogenic, mammogenic, growth), androgens, corpus luteum hormones, estrogens, adrenal cortical hormones, and thyroid substances.

The chapter dealing with statistical methods should be of interest and help in the design and plan of various assays. While it might have been desirable to have contributors present more examples of procedures used by them and also to give a somewhat more detailed evaluation of the various procedures discussed, this would have necessitated a considerable increase in the size of the volume. Nevertheless, this text will be of great value and assistance, both in a practical and theoretical sense, to those investigators concerned with the identification, activity, and quantitative estimation of various hormones.

CHEMISTRY VISUALIZED AND APPLIED. By Armand Joseph Courchaine. New York, G. P. Putnam's Sons, 1950. xiv + 687 pp. $5.50.

This monograph is designed for those entering a career related to health and disease and is written on the supposition that no one using the text will have had any previous preparation in chemistry. It includes three parts—inorganic, organic, and biological chemistry.

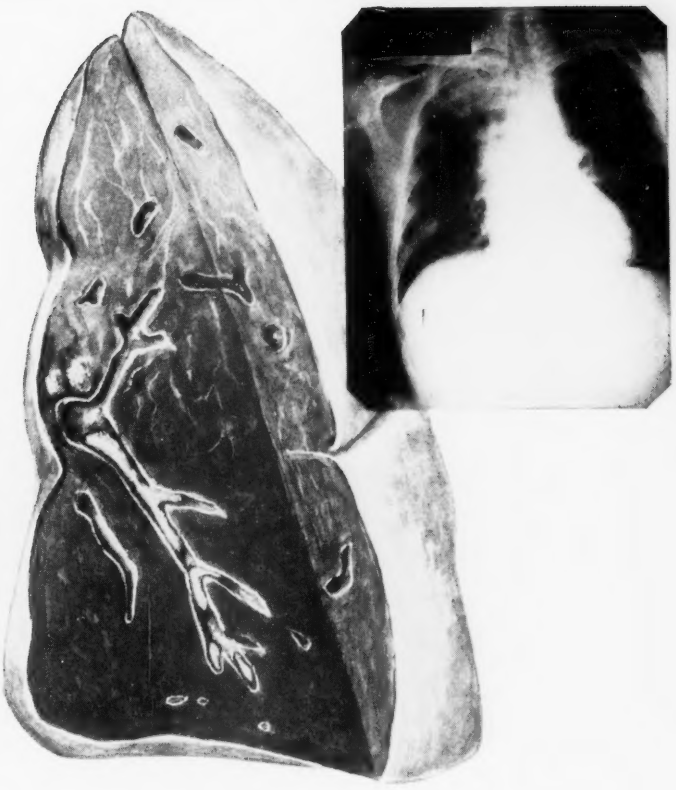
The fundamentals of inorganic chemistry, including pH and ionization, are encompassed in twelve short chapters. Only the most important classi-

fications of organic compounds are found in part two, and an attempt has been made to relate them and their structure to biological chemistry sections following. Part three, on biological chemistry, sketches enzymes, digestion, metabolism, fats, carbohydrates, and amino acids. Some reference is made to the chemistry of blood, milk, and urine. The chapter on hormones lists each hormone plus some amplification. Throughout, only the salient facts of composition, identification, and reactions of compounds are emphasized.

The author apparently draws on his experience as instructor in biological chemistry, science instructor, chemical analyst, and laboratory supervisor for the major part of his source material. In some chapters he refers to secondary material taken from standard textbooks.

The book is well organized and is easily read. The use of diagrams, structural formulae, sketches, and photographs is adequate. Introductory questions at the beginning of each chapter and sample test questions at the end are of value to the beginner. Some provision has been made for the use of portions of each chapter as a "short" course or of the complete book as a survey course for college undergraduates. This text might be of value in such a survey course or possibly in nursing education.

J. K. R.



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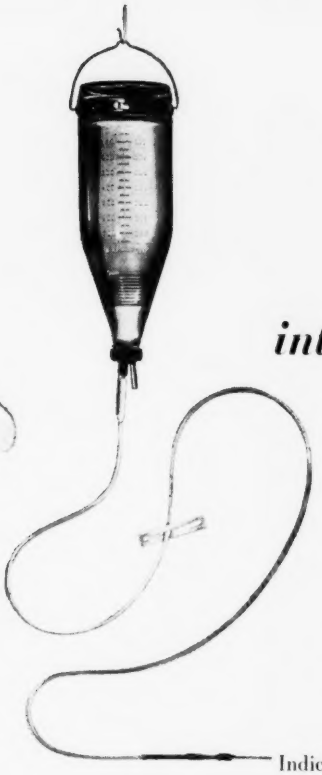

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